IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)
	)
NI *et al.*	) Confirmation No. 1025
	)
Application Serial No. 10/652,622	) Group Art: 1635
	)
Filed: August 29, 2003	) Examiner: Schneizer, Richard
	)
FOR: "DELIVERY OF PHYSIOLOGICAL AGENTS WITH *IN-SITU* GELS COMPRISING ANIONIC POLYSACCHARIDES"	)

DECLARATION UNDER 37 C.F.R. § 1.132 OF YAWEI NI, Ph.D.

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Commissioner for Patents
P.O. Box 1450
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Customer Number 23859

Sir:

I, Yawei Ni, offer the following declarations as evidence in the prosecution of the captioned patent application, under the provisions of 37 C.F.R. § 1.132:

1. I received a PhD. from the Department of Veterinary Microbiology and Parasitology from Texas A&M University in 1991, served as a Post Doctoral associate at the Division of Molecular Biology of Baylor College of Medicine in Houston Texas in 1992, as a Post Doctoral Research Associate at the Department of Veterinary Pathobiology at Texas A&M University from 1993-1995, as a Research Scientist, Project Leader , and/or Team Leader for Discovery, Chemistry, and Drug Delivery at Carrington Laboratories of Irving Texas from 1993-2002, and in 2003 I was promoted to Research Fellow, then Senior Scientist at Carrington's DelSite Biotechnologies Inc. subsidiary in 2003 until 2007, responsible for Discovery Research and Vaccine Development. I was recently promoted to Chief Scientific Officer at DelSite Biotechnologies, where I continue to supervise DelSite's programs in Vaccine and Therapeutics Development. My Curriculum Vita is attached hereto in Exhibit A.

2. The original claims of the above-referenced patent application Serial No. 10/652,622 are currently under rejection in a Final Office Action dated June 25, 2007, all of which documents I have read and understood. I understand that this Declaration will be used in a Response to that Final Office Action, to be filed in conjunction with a Request for Continued Examination.

3. The Office Action rejects all of the claims as being unpatentable for obviousness under 35 USC § 103, over various references, including Baichwal (U.S. Patent No. 5,612,053), Watts (U.S. Patent No. 6,310,089), and Ni (U.S. Patent No. 5,929,051), as well as others. On pages 10-12, the Office Action rejects previous arguments that multiple selections from the various references were required to produce a hypothetical composition "wherein the composition is not pre-gelled as formulated, and having appropriate particle sizes wherein the particles would form a gel in situ. This is unpersuasive because it is presented as an opinion and not supported by any evidence." The Examiner made similar statements requesting evidence as will be further described below.

4. In this Declaration I offer evidence of Longstanding but Unmet Needs in the Art, and/or results of laboratory experiments providing evidence of unexpectedly superior results in the delivery of vaccines, as a response to the Examiners' rejections and statements.

5. My research activities over the years included much experience in drug delivery, polysaccharide chemistry, manufacturing, formulation development, preclinical studies in animal models, and clinical studies. I was a primary inventor of Aloe pectins, a chemically and functionally distinct class of pectins that are unexpectedly superior gelling agents, and later co-discovered the inventions disclosed in this application, namely that solid pharmaceutical compositions comprising a low percentage of pectins having a combination of low degrees of methoxylation and high molecular weights are capable of stabilizing sensitive biological active agents in pharmaceutical formulations, and can form gels on mucosal surfaces "in-situ," in the presence of the very low concentrations of calcium ions found in biological fluids, allowing such formulations to provide unexpectedly superior performance in the delivery of drugs to mucosal surfaces. This "in-situ gelling" phenomenon is extremely useful in a wide range of applications in the wound care and drug delivery, so as to satisfy long felt but unmet needs in the practical administration of many active agents, for example, in the delivery of vaccines.

6. Safe and effective vaccines (currently all liquids) are readily available and typically widely administered by injections in the economically developed world, and currently save many lives

by providing immunization against many communicable diseases. Nevertheless, liquid vaccines and their delivery technologies are often impractical and/or significantly deficient when their use is attempted in many developing countries. As noted by the World Health Organization and UNICET in their "Global Inmmunization Vision and Strategy 2006-2015," more than 27 million infants and 40 million pregnant women worldwide remain in need of immunization (see page 9 of **Exhibit B),** and "Global interdependence has increased the vulnerability of people everywhere to the uncontrolled spread of disease though epidemics," (see page 3, **Exhibit B).**

7. As noted by Myron M Levine of the University of Maryland School of Medicine, on page 99 in the article attached in **Exhibit C,**

> "In developing countries, delivery of immunization would be more efficient and economical if all vaccines were temperature stable, required less than three doses to immunize, and could be administered without needles. However, all EPI vaccines are now given using needles and syringe. This is problematic because in developing countries injection safety is a notorious problem: improper practices involving nonsterile needles and syringes (often re-used from one person to another) cause abscesses and transmit blood-borne pathogens (such as Hepatitis B and HIV)."

Levine also notes that:

> "In developed countries, immunization without needles or syringes would increase acceptability (and therefore compliance) and would enhance occupational safety for vaccinators and other health providers. This could be particularly critical in the future should it become necessary to immunize large populations rapidly en masse in the face of a pandemic influenza or bio-terror emergency."

8. The problems with the requirement of current vaccines for administration by injection is greatly exacerbated by the temperature/storage instability of current vaccine formulations, and their corresponding requirement for cold storage and/or or cold chain management. See for example the article by Obaro and Palmer of **Exhibit D** "There has been no formal system in place for

continuous evaluation and replacement of refrigerators and solar panels. Consequently, there are frequent breaks in the cold chain and as this often goes unnoticed, may be responsible for vaccine failures and loss of public confidence in vaccines in some settings. The strengthening of the existing cold chain infrastructure, a costly investment, is a major challenge for developing countries..." see page 1425 of **Exhibit D.**

9. Even if cold chain management issues can be addressed at major urban centers in developing countries, many rural areas are often inaccessible by road or other reasonable modern means, so that local distribution and administration of vaccines in those rural areas is often problematic, as can be graphically illustrated by the picture on page 58 of **Exhibit B,** which shows a picture of one of the current methods for local distribution and administration of vaccines in rural areas of the developing world, namely a health worker riding a motorcycle while carrying a Styrofoam cold box for carrying the current liquid vaccines and boxes of syringes and syringe disposal equipment to use in local administration of the vaccines.

10. Given the state of affairs described above and in the Exhibits it is clear that a "Long Felt But Unmet Need" still exists for vaccine formulations that are storage and/or temperature stable, and that do not require administration via injection. The nasal powder vaccine compositions disclosed and claimed in this application meet those "Long Felt But Unmet Needs", as described below, a result that is legally effective evidence of non-obviousness.

11. As evidenced by the evidence and laboratory data results described below, the nasal powder vaccine compositions encompassed in the current claims meet those needs by providing powder compositions that are temperature and storage stable, and are administered by simply applying the powders to the nasal cavity by insufflation, wherein they form "in-situ" gels that provide superior adherence of the gels to the nasal mucosa and release the vaccine antigens to contact with the mucosa in a time-extended fashion, so as to provide for unexpectedly superior induction of an immune response in the animals.

12. Powder vaccine compositions falling within the scope of the claims of this application were prepared having the compositions described in Tables 1 and 2 below:

Table 1. Nasal powder formulations		
Components	**Liquid % w/v**	**Powder % w/w**
Antigen	0.001 – 0.05	0.01 – 0.5
Aloe pectin polymer	0.01 – 0.1	0.1 – 1
Povidone (K29-32)	0.05	~0.5
Buffering agents/salts	0.21	~2.1
Lactose monohydrate	10	> 96

Table 2. Virus strains and antigens used in the preclinical studies.	
Type and subtype	**Strain**
H1N1	A/New Caledonia/20/99
H1N1	A/Taiwan/1/86
H3N2	A/Wyoming/03/2003
B	B/Jiangsu/10/2003)
H5N3	A/Duck/Singapore-Q/F110-2/97

The pectin used in the nasal powder formulations was obtained by extraction of fibers isolated from Aloe vera leaves, using EDTA as described in the specification of this application, and had a galacturonic acid content of greater than 90% and a degree of methylation of less than 10%. The powder compositions according to Table 1 were prepared by dissolving the components in water to form a liquid formulation having a pH of 7.0-7.4, then spray drying 30-50 ml of the liquid formulations using a Buchi 290 spray dryer, with conditions adjusted to produce mean particle sizes of greater than 20 μm. With the use of ultrasonic nozzles, powders with a narrow particle size distribution as characterized by a Span [(D90-D10)/D50] of ~1.5 were produced. The moisture content of the powders was determined by thermogravimetric analysis (TGA) and found to be consistently between 3% and 6%. The recovered yields of powder were approximately 60%, the losses being attributed to irrecoverable coating losses to the vessel walls by the powder compositions at these small scales. Alternatively, powders were prepared by lyophilization-milling method. The same liquid formulation was placed into 20 ml lyophilization vials (10 ml/vial), and freeze-dried (lyophilized) in LyoStar II (FTS system, New

York). The dried materials were milled using a ball mill (Retsch MM301) followed by sieving using 40 and 100 μm sterile nylon membranes under vacuum to produce powders of <40 μm, 40 – 100 μm, and >100 μm. Powders of the 40 – 100 μm size were used in animal studies. The powder compositions were stored in an electronic dessicator cabinet. The particle size distribution was determined using a Beckman Coulter LS13 320 laser diffraction particle size analyzer.

13. The resulting powders quickly and completely dissolve in pure water, and were assayed for antigen stability over periods of time up to two years (Table 3, after storage at room temperature in dessicator cabinets), by measuring the HA antigen activity of the powders using chicken red blood cells in V-bottom 96 well plates by the method of Barrett and Inglis (Virology, a Practical Approach, pg 119-150, IRL Press, Oxford 1985 , W.J. Mahy Editor). No loss of HA activity was observed during the process of preparing the powders.

14. As can be seen from Table 3, the HA antigen activity of the powders was completely stable over the 24 months tested. Moreover the HA activity of the nasal powders comprising pectins was significantly higher than that of control compositions that did not comprise the small amount of pectin, indicating that the Aloe pectin has a stabilizing effect on the vaccine antigens.

Table 3. Antigen stability of liquid and powder formulations by freeze drying method during storage at ~22 °C

Formulations	Antigen content*	HA activity at different time points (months)						
		0		3	8	12	18	24
		Liqd	Pwd	Pwd	Pwd	Pwd	Pwd	Pwd
No Antigen	0 μg/mg	0	0	0	0	0	0	0
No Pectin	0.26 μg HA/mg	1024	512	512	512	512	512	512
Pectin/Antigen 1x	0.26 μg HA/mg	1024	1024	1024	1024	1024	1024	1024
Pectin/Antigen 2x	0.52 μg HA/mg	2048	2048	2048	2048	2048	2048	2048

* Total protein (μg) / powder (mg). Antigen (A/Taiwan/1/86 strain whole virion).

15. As previously noted the powder particles quickly and completely dissolve in pure water, but they do not dissolve in simulated nasal fluid comprising calcium at the concentration observed in human nasal fluids (~ 5 mM calcium), instead forming insoluble gel particles that swell to approximately twice their initial size, illustrating the "In-Situ Gellation" property of

formulations. As can be seen in Figure 1 below, this in-situ gellation property of the powders comprising Aloe pectins slows the release of the vaccine antigens into solution. If no pectin is present, the antigens are immediately released into solution, but increasing amounts of pectin slow release of the antigen into solution.

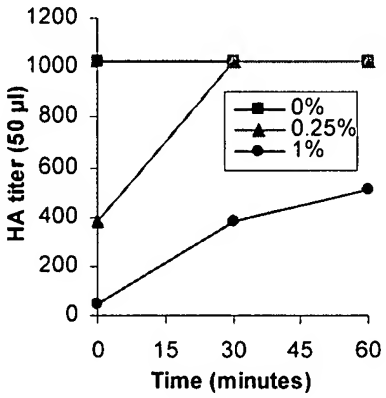


Fig. 1. Sustained antigen release from powder formulations containing avian H5N3 whole virion antigen with different Aloe pectin polymer contents (% ,w/w). Dried powder formulations were mixed with simulated nasal fluid and the fluid sampled at different time points for HA antigen activity.

16. The ability of the powder formulation comprising Aloe pectins to gel "in-situ" was also demonstrated "in-vivo" by applying the powder formulations to the nasal mucosal surfaces of rats. As can be seen in Figure 2, powder formulations with a particle size of 40 -100 µm were delivered intranasally to rats, then the rats were sacrificed, and their nasal cavities examined by histological staining (Toluidine blue) of cross sections. The powder particles formed purplish thin sheets of gel material that were observable on the surface of nasal epithelium up to 5 hrs. This contrasts with the normal rapid clearance of materials the mucosal surface (see Exhibit E, pages 28-29). A normal mucocilliary clearance time has been estimated to be 12-15 minutes in humans (see Exhibit F, page 38).

17. Subsequent animal testing confirmed the excellent effectiveness of the powder compositions comprising Aloe pectins for stimulating immunity after nasal administration. In one set of experiments, powder formulations employing the A/Taiwan/1/86 (H1N1) whole virion antigen and containing Aloe pectin polymer at two concentration were compared with control formulation not containing the pectin. (See Table 4).

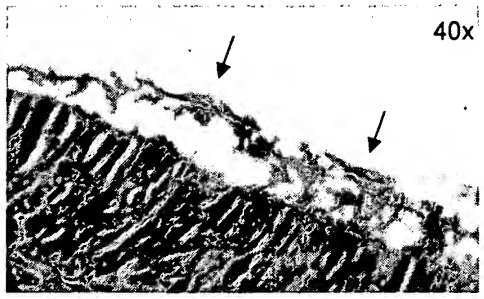


Figure 2. Gel sheets on rat nasal epithelium formed by Aloe pectin containing powders after 5 hours.

Table 4. Effect of Aloe pectin - HAI titers [GMT (SD)]					
Parameters		**Groups [n=5 (rats)]**			
		No Antigen	**Antigen = (A/Taiwan/1/86 whole virion)**		
			Pectin 0%	**Pectin 0.5%**	**Pectin 1.0%**
Antigen dose(μg HA/rat)		0	7.1	7.1	7.1
Powder dose(mg/nostril)		8	8	8	8
Pectin content (%, w/w)		1	0	0.5	1
Route/No. nostrils		IN/2	IN/2	IN/2	IN/2
Time after one inoculation	2w	5 (0)	**11** (32)	**56** (78)	**33** (28)
	4w	5 (0)	**40** (59)	**226** (247)	**269** (301)
	6w	5 (0)	**26** (28)	**160** (136)	**160** (275)

18. The results showed that the presence of pectin significantly and unexpectedly increased the immune response ($p < 0.05$; Table 4). No apparent difference in HAI titers was observed between the two GelSite polymer contents (0.5% and 1%) (Table 4).

19. Subsequently, a wider range of GelSite polymer content (0.1% - 1%, w/w) was tested using an H5N3 antigen (Table 5).

Table 5. Optimal Aloe pectin content – HAI titers [GMT (SD)]							
Parameters			Groups [n=5 (rats)]				
			No Ag	Antigen = (Avian H5N3 whole virion)			
				0.10%	0.25%	0.50%	1.00%
Antigen dose(μg HA/rat)			0	3.3	3.3	3.3	3.3
Powder dose(mg/nostril)			6.5	6.5	6.5	6.5	6.5
Pectin content (w/w %)			1	0.1	0.25	0.5	1
Route/No. Nostrils			IN/2	IN/2	IN/2	IN/2	IN/2
Time after inoculation	1st	2w	5 (0)	53 (28)	60 (22)	40 (30)	23 (9)*
		4w	5 (0)	139 (36)	121 (44)	105 (57)	60 (22)*
	2nd	2w	5 (0)	367(143)*	557(143)	422 (226)	183(132)*
		4w	5 (0)	640 (351)	844(351)	422(175)*	320(243)*
		6w	5 (0)	844 (858)	970 (350)	557 (143)	442(175)*
* Significantly lower ($p< 0.05$) compared to the titer at 0.25% at the same time point.							

20. The results showed that the nasal powders containing the pectin at 0.25% (w/w) typically induced the highest HAI titers, suggesting that in formulations development, Aloe pectin concentration should be optimized, most likely in order to optimize the release rate of antigen so as to produce maximal immune response.

21. Overall, in my expert opinion, the data reported above indicate that the inclusion in nasal vaccine powder compositions of pectins comprising a low degree of methylation, such as the Aloe pectins used in the studies above, meet a previously longfelt but unmet need for vaccine composition that can be stored for long periods of time at room temperature without loss of activity, and that when administered to nasal mucosa without a need for injection, to produces a unique "in-situ" gel on the nasal membranes that stabilizes and extends the period of contact between the vaccine antigens and the nasal mucosa, so as to produce unexpectedly superior immunological responses.

22. Lastly, I would like to address one statement by the Examiner in the Office Action, which I regard as erroneous. On page 12 of the Office Action, the Office Action states that "the dry compositions of Baichwal (U.S. Patent No. 5,612,053) which absorb water and form gels (Column 8, lines 22-25) ...." I find no such disclosure at Column 8, lines 22-25. I did find a disclosure at Baichwal in Column 6, lines 25-28, that the starches that are one of the many alternative polysaccharide gums taught by Baichwal to be suitable in his invention can be "gelled or ungelled," but I find the Examiner's point to be a misinterpretation of Baichwal. It

is certainly true that some polysaccharides (including starches and pectins) can form gels, by various mechanisms, under various conditions that are specific to each polymer. Nevertheless, the fact that a given polysaccharide is capable of forming a gel under some specified conditions has very little to do with the completely separate and much more difficult question of whether a particular polysaccharide will, when it is initially ungelled and then formulated into a pharmaceutical composition, will then gel "in-situ" upon application to a mucosal surface. In my opinion, it is erroneous and misleading to infer that because Baichwal disclosed the known fact that certain starches can be gelled, that such a simple fact in any way suggests that such starches, or even other polysaccharides such as pectins, will gel in-situ.

23. In a similar way, I disagree with any contention that either the Watts, Ni or other references cited by the Examiner suggest the formation of, or utility of "in-situ" gel formation on contact with mucosal surfaces with claimed composition. Accordingly, I do not believe that any of the references cited by the examiner in the Office Action, taken alone or together, suggested the unique and invaluable property of "in-situ gellation" possessed by the claimed compositions, and that therefore the claimed compositions are unobvious.

24. Accordingly, in my opinion, in view of the discussion, articles, and laboratory data and evidence included herein, the vaccine compositions encompassed by the current claims, are not obvious over the prior art cited by the examiner.

25. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements, perjury, and the like so made are punishable by fine or imprisonments, or both, under Section 1001 of Title 18 of the United States Code and that any such willful false statement or perjury may jeopardize the validity of the application or any patent issued thereon.

Yawei Ni, Ph.D.

31 Oct 2007
Date

EXHIBIT A

RESUME OF YAWEI NI, PHD

RESUME OF YAWEI NI, PHD

NAME	POSITION TITLE
Ni, Yawei	Chief Scientific Officer

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Yunnan Agriculture University, Yunnan, China	BVM	1982	Veterinary Medicine
National Institute of Veterinary Bioproducts and Pharmaceuticals/Beijing University,Beijing, China	M.S.	1985	Microbiology & Immunology
Texas A&M University, College Station, Texas	Ph.D	1991	Microbiology
Baylor College of Medicine, Houston, Texas	Postdoctoral	1992	Virology
Texas A&M University, College Station, Texas	Postdoctoral	1993	Immunology and Cell Biology

A. Positions and Honors.

Positions and employment

1985-1987	Research Associate, National Institute of Veterinary Bioproducts and Pharmaceuticals, Beijing, China.
1987-1991	Research Assistant (Ph. D student), Department of Veterinary Microbiology and Parasitology, Texas A & M University, College Station, Texas.
1991-1992	Postdoctoral Research Associate, Division of Molecular Virology, Baylor College of Medicine, Houston, Texas.
1992-1993	Postdoctoral Research Associate, Department of Veterinary Pathobiology, Texas A&M University, College Station, Texas.
1993-1995	Research Scientist, Department of Veterinary Pathobiology, Texas A&M University, College Station, Texas.
1995-1998	Research Scientist, Carrington Laboratories, Inc. Irving, Texas.
1998-2000	Research scientist, Project leader, Carrington Laboratories, Inc. Irving, Texas.
2000-2002	Research Scientist, Team leader (Discovery, chemistry, and drug delivery), Carrington Laboratories, Inc. Irving, Texas.
2002-2003	Research Fellow, Group Leader for Discovery Research, DelSite Biotechnologies Inc (a subsidiary of Carrington Laboratories Inc).
2003-2006	Research Fellow, Group Leader for Discovery Research and Vaccine Development, DelSite Biotechnologies Inc (a subsidiary of Carrington Laboratories Inc).
2006-2007	Senior Scientist, DelSite Biotechnologies Inc (a subsidiary of Carrington Laboratories Inc).
2007-now	Chief scientific officer, DelSite Biotechnologies Inc (a subsidiary of Carrington Laboratories Inc).

Other Experience and Professional Memberships

2005-present	Interscience Conference on Antimicrobial Agents and Chemotherapy
2000-present	Member of Controlled Release Society
2002-present	Member of American Association of Pharmaceutical Scientist
1995-present	Member of Glycoscience Interest Group
1990-1995	Member of American Society of Virology
1985-1986	Member of the study section on viral diarrhea, Institute of Virology, Health Ministry, Beijing, China.

Honors

1982	Honor student award, Yunnan Agriculture University, China.
1982	National fellowship for graduate studies, Agriculture Ministry, Beijing, China.
1985	Outstanding thesis research award, National Institute of Veterinary Bioproducts and Pharmaceuticals, Beijing, China.
1994	Service Award, Department of Veterinary Pathobiology, College of Veterinary Medicine, Texas A&M University.

B. Selected peer-reviewed publications (in chronological order).

1. Ni, Y. (1986). Isolation and characterization of bovine and porcine rotaviruses. J. Virol.(Beijing) 2, 36 - 41.
2. Ni, Y. (1986). Detection of porcine and bovine rotavirus antigens and antibodies by NT, ELISA and RNA electrophoresis. J. Virol.(Beijing) 2, 255 - 259.
3. Ni, Y., Qiu, H., Liao, G., Zhu, Y., and Zhou, T.(1987). Genome reassortment of porcine rotaviruses and the isolation of a porcine rotavirus with short RNA pattern. Commun. Chinese Vet. Bioprodcuts and Pharmaceuticals 2, 35-38.
4. Ni, Y., Qiu, H., Zhu, Y., and Zhou, T. (1989). Isolation and characterization of a small round virus about 15 nm in diameter from the feces of a piglet with diarrhea. ACTA Veterina et Zootechnica Sinica, supplement, 174-178.
5. Ni, Y., Qiu, H., Zhu, Y., and Zhou, T.(1989). Molecular epidemiology investigation of porcine rotaviruses in Beijing area. Chinese J. Vet. Med. 15, 11-12.
6. Haddow, J., Clark, B., Ni, Y. and Desselberger, U (1989). Biological function of the rotavirus protein VP4: observation on porcine isolates from China. Med. Microbiol. Immunol. 178,163-167.
7. Clark, D. F., Ni, Y., Collisson, E. W., and Kemp, M. C. (1990). Characterization of avian reovirus strain-specific polymorphisms. Avian Dis. 34, 304-314.
8. Ni, Y. and Kemp, M. C. (1990). Selection of genome segments following coinfection of chicken fibroblasts with avian reoviruses. Virology 177, 625 -633.
9. Kahlon, J. B., Kemp, M. C., Ni, Y., Carpenter, R. H., Shannon, W. M., and McAnally, B. H. (1991). In vitro evaluation of the synergistic antiviral effects of acemannan in combination with azidothymidine and acyclovir. Mole. Biotherapy 3, 214-223.
10. Ni, Y. and Kemp, M. C. (1992). Strain-specific selection of genome segments in avian reovirus coinfections. J. Gen. Virol. 73, 3107 - 3113.
11. Ni, Y., Ramig, R. F. and Kemp, M. C. (1993). Identification of proteins encoded by avian reoviruses and evidence for posttranslational modification. Virology 193, 466 - 469.
13. Ni, Y. and Ramig, R. F. (1993). Characterization of avian reovirus-induced cell fusion: the role of viral structural proteins. Virology 194, 705 - 714.
14. Ni, Y. and Kemp, M. C. (1994). Subgenomic S1 segments are packaged by avian reovirus defective interfering particles having an S1 segment deletion. Virus Res. 32, 329-342.
15. Ni, Y. and Kemp, M. C. (1995). A comparative study of avian reovirus pathogenicity: virus spread/replication and induction of lesions. Avian Dis. 39, 554-566.
16. Ni, Y., Kemp, M. C., and Ramig, R. F. (1996). Genetic determinants of avian reovirus pathogenesis. Proceedings of international symposium on adenovirus and reovirus infections in poultry, Rauischholzhausen, Germany.
17. Ni, Y., Kemp, M. C., Wang, L., and Collisson, E. W. (1996). Sequence analysis of an avian reovirus DI RNA-identification of sequence signals involved in replication and packaging. Proceedings of international symposium on adenovirus and reovirus infections in poultry, Rauischholzhausen, Germany.
18. Ni, Y. and Tizard, I. (1996). Lectin-carbohydrate interaction in the immune system. Vet. Immunol. Immunopathol. 55, 205-223.
19. Tizard, I and Ni, Y. (1998). Use of serologic testing to assess immune status of companion animals. J. Am. Vet. Med. Assoc. 213, 54-60.
20. Sirinivansan, A., Ni. Y., and Tizard, I. (1999). Specificity and prevalence of natural bovine anti-mannan antibodies. Clin. Diagn. Lab. Immunol. 6, 946-952.

21. Ni, Y., Powell, R., Pather, D. and Tizard, I. (2000). Specificity and prevalence of bovine natural anti-alpha galactosyl (Gal α1-6/Gal/Glc) antibodies. Clin. Diagn. Lab. Immunol. 7, 490-496.
22. Zhang, Z., Guo, J., Ni, Y., Bazer, F. W., Giavedoni, L., and de la Concha-Bermejillo, A. (2003). Construction and characterization of a recombinant ovine lentivirus carrying the optimized green fluorescent protein gene at the dUPTase locus. Arch. Virol. 148, 1485-1506.
23. Ni, Y., Turner, D., Yates, K. M., Tizard, I. (2004). Isolation and characterization of structural components of Aloe vera L. leaf pulp. Int Immunopharmacol. 4,1745-1755.
24. Ni, Y., Turner, D., Yates, K. M., Tizard, I. (2007). Stabilization of growth factors related to wound healing with a plant cell wall biomaterial. 4,1745-1755.

Book Chapters

1. Tizard, I and Ni. Y. (1998). Immunostimulating effect of carbohydrates. *In* Encyclopedia of Immunology, Delves, P. J. and Roitt, I. M (eds.). 2nd ed. Vol 1. P427-431. Academic Press. San Diego.
2. Ni, Y.and Tizard, I. (2004). Analysis of *Aloe* Pulp and Its Derivatives. *In* Medicinal and aromatic plants-industrial profiles, Aloes. T. Reynolds (ed.), P111-126, CRC press. Boca Raton.
3. Ni, Y., Yates, K. M., and Tizard, I. (2004). Aloe polysaccharides. *In* Medicinal and aromatic plants-industrial profiles, Aloes. T. Reynolds (ed.), P 75-87, CRC press, Boca Raton.

Patents

1.	7,202,066	Combination of a growth factor and a protease enzyme
2.	7,022,683	Pharmacological compositions comprising pectins having high molecular weights and low degrees of methoxylation
3.	6,777,000	In-situ gel formation of pectin
4.	6,313,103	Pectic substance as a growth factor stabilizer
5.	6,274,548	Pectic substance as a growth factor stabilizer
6.	5,929,051	Aloe pectins

C. Research Support.

Ongoing Research Support

1. 1-R43-AI058440, Ni (PI), 03/01/04-02/28/06 (Completed)
   NIAID
   An in-situ gelling nasal vaccine delivery platform.
   This project is to develop an effective nasal vaccine delivery platform based on a unique in-situ gelling polymer (GelSite).
   Role: PI

2. 1 UC1 AI62511-01, Ni (PI), 9/30/2004 – 08/31/2008
   NIAID
   An inactivated influenza nasal powder vaccine
   This project is to develop an inactivated influenza nasal powder vaccine.
   Role: PI

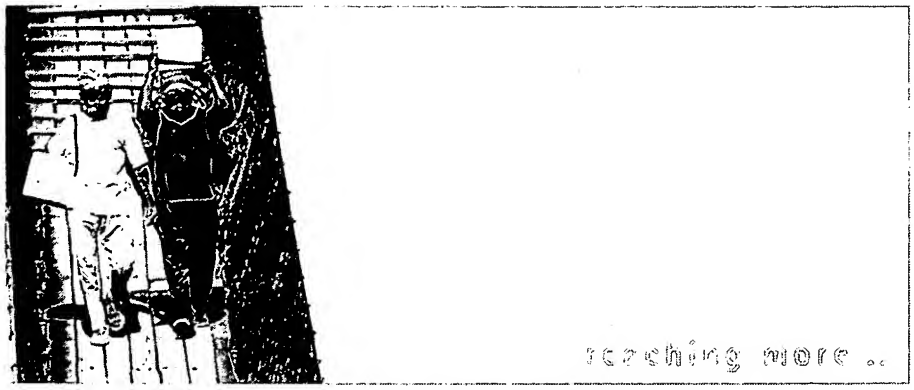
3. Evaluation of GelSite polymer for Intranasal vaccine delivery. 2002-present.
   This project evaluated the in-situ gel formation of GelSite polymer in the nasal cavity and its potential use for nasal vaccine delivery. It has progressed smoothly from demonstration of gel formation in the nasal cavity to the increased immune response following delivery with the GelSite polymer. The results of this project showed that the GelSite polymer potentially constitutes a simple and broad nasal vaccine delivery platform.
   Delsite Biotechnologies Inc (a subsidiary of Carrington Laboratories Inc).

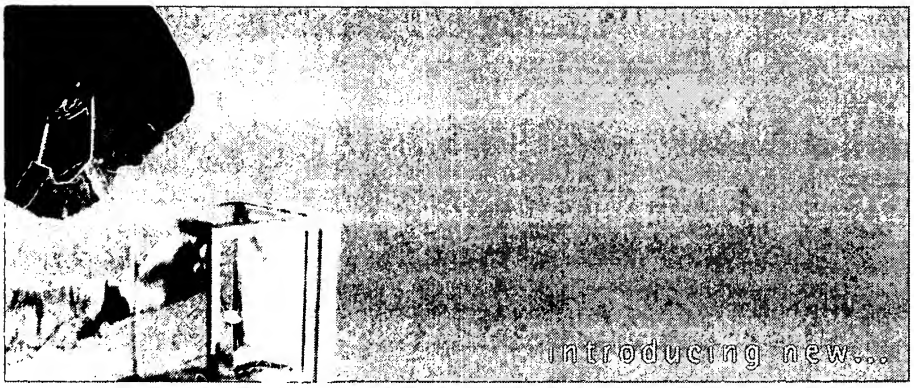
Role: Principal investigator

4. In-situ gelation of GelSite polymer and drug delivery. 1998-present.
This project examined the in-situ gel formation of GelSite polymer following administration via various routes, various factors affecting the in-situ gelation, and the use of GelSite polymer for drug delivery, especially topical protein delivery.
DelSite Biotechnologies Inc.
Role: Principal investigator.

EXHIBIT B

"Global Immunization Vision and Strategy 2006-2015,"
Jointly formulated and distributed by the World Health Organization and UNICEF





GIVS

Global Immunization Vision and Strategy 2006–2015

World Health Organization

unicef

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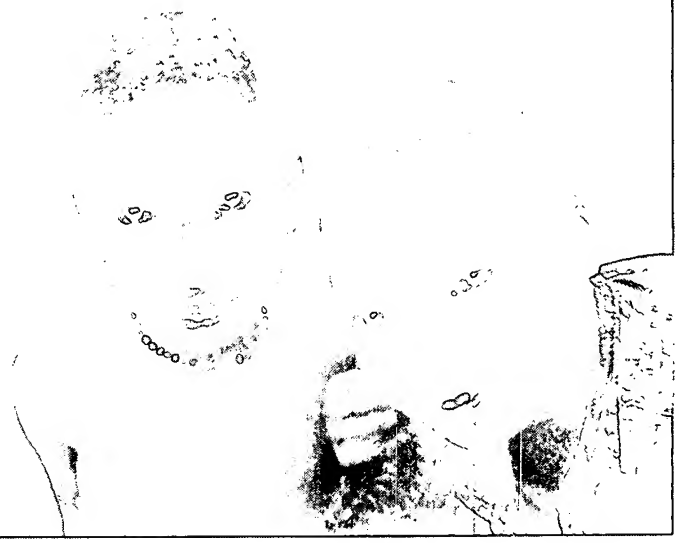

GIVS

Global Immunization Vision and Strategy
2006-2015

Contents

Foreword

Immunization is one of the most successful and cost-effective health interventions ever. It has eradicated smallpox, lowered the global incidence of polio so far by 99% and achieved dramatic reductions in illness, disability and death from diphtheria, tetanus, whooping cough and measles. In 2003 alone, it is estimated that immunization averted more than 2 million deaths.

Immunization has a promising future. We are entering a new era in which it is expected that the number of available vaccines will double. Immunization services are increasingly used to deliver other important health interventions, making them a strong pillar of health systems.

Immunization will help to achieve the Millennium Development Goals on reducing child mortality, improving maternal health and combating diseases, eventually including malaria and HIV/AIDS.

In spite of its undisputed past success and promising future, however, immunization remains an unfinished agenda.

We are alarmed that globally and in some regions immunization coverage has increased only marginally since the early 1990s.

There are still millions of people who do not benefit from the protection that vaccination provides. They are at risk of life-threatening illness every day. An estimated 27 million infants and 40 million pregnant women were not immunized in 2003. Approximately 2.5 million children under five years of age die every year as a result of diseases that can be prevented by vaccination using currently available or new vaccines.

Together we can and will change these sobering statistics.

Our mission is to build on past achievements and use our know-how and experience to save more lives. This Global Immunization Vision and Strategy guides countries on how to immunize more people against more diseases; introduce newly available life-saving vaccines and technologies; and provide other critical health interventions (e.g. nutrition and malaria control) at immunization contacts.

Global interdependence has increased the vulnerability of people everywhere to the uncontrolled spread of diseases through epidemics. The mounting threat of an influenza pandemic highlights the need to strengthen international solidarity, mutual support and work through partnerships to contribute to improving global health and security.

In spring 2005, the Member States of WHO and the Executive Board of UNICEF approved this Global Immunization Vision and Strategy. The Strategy will enable global stakeholders to address the serious challenges foreseen in immunization over the next decade: these include financing new and underused vaccines, ensuring adequate supply and access for all people who require and deserve the protection of vaccines, whether rich or poor.

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WHO and UNICEF would like to thank all the people who have been involved in the development of the GIVS, for their vision, expertise and hard work.

Executive summary

A new vision for immunization

In response to the challenges of a rapidly changing and increasingly interdependent world, WHO and UNICEF have jointly drafted this global immunization vision and strategy for the years 2006–2015. Its goal is to protect more people against more diseases by expanding the reach of immunization to every eligible person, including those in age groups beyond infancy, within a context in which immunization is high on every health agenda. It aims to sustain existing levels of vaccine coverage, extend immunization services to those who are currently unreached and to age groups beyond infancy, introduce new vaccines and technologies, and link immunization with the delivery of other health interventions and the overall development of the health sector. It places immunization firmly within the context of the health system, highlighting the fact that immunization can both benefit from – and contribute to – the development of the health sector and to overcoming system-wide barriers. The strategy also underlines the crucial contribution of immunization to global preparedness for epidemics and complex emergencies. The realization of this vision of immunization will need strengthened surveillance, monitoring and evaluation, and the application of solid data for programme management.

Guiding principles

The following guiding principles have inspired the formulation of the global strategy.

Equity and gender equality
All people – without distinction of race, religion, political belief, economic or social condition – have a right to equal access to the needed vaccines and interventions.

Ownership, partnership and responsibility
Goals are commonly agreed and pursued by governments and their partners, joined by international solidarity, which engage in coordinated activities determined by national plans.

Vision: A world in 2015 in which:

- *immunization is highly valued;*
- *every child, adolescent and adult has equal access to immunization as provided for in their national schedule;*
- *more people are protected against more diseases;*
- *immunization and related interventions are sustained in conditions of diverse social values, changing demographics and economies, and evolving diseases;*
- *immunization is seen as crucial for the wider strengthening of health systems and a major element of efforts to attain the Millennium Development Goals;*
- *vaccines are put to best use in improving health and security globally; and*
- *solidarity among the global community guarantees equitable access for all people to the vaccines they need.*

Accountability
Stakeholders and actors in immunization are publicly accountable for their policies and actions.

Assured quality and safe products and services
All products made available meet internationally recognized standards of quality and safety, and services are delivered according to best practices.

Strong district-based immunization systems
Interventions and their monitoring at district level ensure local commitment and ownership and the appropriate adaptation of the programme to local needs and circumstances.

Sustainability through technical and financial capacity building
Financial and technical self-reliance is a target for national governments and partners working collectively, with continuing, incremental infrastructure building.

Policies and strategies based on evidence and best practices
The choice of policies, strategies and practice is informed by data from operational research, surveillance, monitoring and evaluation, disease burden and impact assessments, and economic analyses, and by the sharing of lessons and experiences from countries in similar circumstances.

Four Strategic Areas

The global strategy comprises four main areas with 24 component strategies. The strategic approaches are: i) protecting more people in a changing world; ii) introducing new vaccines and technologies; iii) integrating immunization, other health interventions and surveillance in the health systems context; and iv) immunizing in the context of global interdependence. Immunization and the other linked interventions described will contribute significantly to the achievement of the Millennium Development Goals, the immunization-related goals set by the United Nations General Assembly Special Session on Children in 2002, and the goals set by the Global Alliance for Vaccines and Immunization and its financing arm The Vaccine Fund. They will also help Member States, as urged in resolution WHA56.19, to increase vaccination coverage against influenza of all people at high risk. In today's increasingly interdependent world, acting together against vaccine-preventable diseases of public health importance and preparing for the possible emergence of diseases with pandemic potential will contribute significantly to improving global health and security.

The global strategy has been drawn up against a background of increasing demand for immunization, rapid progress in the development of new vaccines and technologies, continuing health-sector development, increasing vulnerability to pandemics and other health emergencies, and expanding opportunities for partnerships.

Goals

Between 2006 and 2015, all those working on immunization and related product development should strive to prevent morbidity and mortality by achieving the following goals and targets.

By 2010 or earlier

- **Increase coverage.** Countries will reach at least 90% national vaccination coverage and at least 80% vaccination coverage in every district or equivalent administrative unit.
- **Reduce measles mortality.** Globally, mortality due to measles will have been reduced by 90% compared to the 2000 level.

By 2015 or earlier *(as the case may be)*

- **Sustain coverage.** The vaccination coverage goal reached in 2010 will have been sustained.
- **Reduce morbidity and mortality.** Global childhood morbidity and mortality due to vaccine-preventable diseases will have been reduced by at least two thirds compared to 2000 levels.
- **Ensure access to vaccines of assured quality.** Every person eligible for immunization included in national programmes will have been offered vaccination with vaccines of assured quality according to established national schedules.
- **Introduce new vaccines.** Immunization with newly introduced vaccines will have been offered to the entire eligible population within five years of the introduction of these new vaccines in national programmes.
- **Ensure capacity for surveillance and monitoring.** All countries will have developed the capacity at all levels to conduct case-based surveillance of vaccine-preventable diseases, supported by laboratory confirmation where necessary, in order to measure vaccine coverage accurately and use these data appropriately.
- **Strengthen systems.** All national immunization plans will have been formulated as an integral component of sector-wide plans for human resources, financing and logistics.
- **Assure sustainability.** All national immunization plans will have been formulated, costed and implemented so as to ensure that human resources, funding and supplies are adequate.

The context

The establishment of strong national immunization services in many countries over recent years has ensured that today more than 70% of the world's targeted population is reached by those services. It is estimated that the vaccinations done in 2003 alone will prevent more than 2 million deaths from vaccine-preventable diseases and an additional 600 000 deaths related to hepatitis B (from liver cirrhosis and hepatocellular carcinoma) that would otherwise have occurred in adulthood among the children immunized in that year.

A baby, held by his mother, is vaccinated by a woman health worker. *Credit: UNICEF*

Despite these achievements, commitment to immunization has not been sustained in all countries. Worldwide in 2003 an estimated 27 million infants and 40 million pregnant women remained in need of immunization. Moreover, beyond infancy, children, adolescents and adults do not yet fully benefit from the protection provided through immunization against diseases from which they are at risk.

Strength through partnerships

In response to immunization needs worldwide, global partnerships, such as the Global Alliance for Vaccines and Immunization, The Vaccine Fund, and the Measles Partnership, have been created in order to attain shared goals. Such partnerships bring together major stakeholders in immunization from the public and private sectors, including the vaccine industry. Initiatives for eradication of poliomyelitis, reducing measles mortality and elimination of maternal and neonatal tetanus have shown that partnerships enable immunization services to be brought to even the most hard-to-reach communities. Through the Global Polio Eradication Initiative, for example, countries have clearly demonstrated the capacity to achieve high vaccination coverage rates and conduct high-performance disease surveillance, even in areas affected by political turmoil or other difficult circumstances. However, accessing hard-to-reach populations on a regular basis and those affected by outbreaks and emergency situations requires specially designed strategies.

New vaccines and technologies

Efforts are under way to develop new vaccines against major infectious diseases (including malaria, HIV/AIDS and tuberculosis). Meanwhile, many other new vaccines and technologies are already licensed or at an advanced stage of development (including rotavirus and pneumococcal vaccines) and other vaccines are readily available but underused. Activities to ensure the safety of immunization are also being implemented (such as the use of autodisable syringes) and the subject is becoming a top priority for countries. During the period 2006–2015, countries may be faced with an unprecedented array of new vaccines and technologies for introduction. To ensure that countries can make rational, evidence-based decisions about the choice of new vaccines and technologies, current gaps in knowledge (including disease burden, the cost-effectiveness of various strategies, and regulatory issues) will have to be filled.

Financing

Immunization is a highly cost-effective and relatively inexpensive health intervention. The overall cost of immunization, however, including the procurement of new vaccines, new vaccine formulations and technologies, is expected to rise sharply in the future. The expansion of vaccination schedules to include new vaccines has greatly increased the amount of resources that need to be mobilized. Although some relief may be obtained over time as the larger amounts of vaccine to be procured lead to greater competition among manufacturers and a reduction in price, experience has shown that it takes several years before increased demand for new vaccines is matched by lower prices. Meanwhile, the rising cost of immunization delivery needs to be added to the cost of vaccines; logistics and labour are becoming more expensive, and the extension of services to populations that are currently not being reached will need additional resources.

Securing the financing for the introduction of new vaccines and increasing coverage with existing vaccines will test all countries and their partners. Ways need to be found to maximize the cost-effectiveness of contacts with immunization services (such as spreading the cost of these contacts across relevant health initiatives) and to strengthen national capability to project financial needs and obtain the required resources. Evidence-based policy decisions will have to be taken on the "affordability" of vaccines in relation to the reduction of disease burden.

Contribution to overcoming system-wide barriers

Increasingly, immunization will help to overcome barriers to equitable health-service delivery and sector-wide development, and will benefit from those efforts. The benefits include better public health and improved efficiency of public health services. Immunization services inevitably experience the constraints that affect the health system as a whole, but they can help significantly in overcoming system-wide barriers through the strengthening of district teams and their capacity to make optimal use of the resources and opportunities available locally. In turn, sector-wide approaches to strengthening cross-cutting areas such as human resources management, financing, logistics, public–private partnerships and information sharing can clearly benefit immunization.

Strong monitoring and surveillance capacity

Over the past decade, considerable progress has been made in establishing systems for monitoring and surveillance of coverage rates and trends of vaccination and its impact on vaccine-preventable diseases, and in using those data for guiding public policy, strategies and programmes. Through extensive and growing laboratory networks, surveillance for poliomyelitis and measles has not only generated crucial information for guiding the respective eradication and mortality-reduction initiatives, but has also supported the prevention and control of epidemics of, for instance, meningitis, diphtheria, rubella and vector-borne diseases such as dengue and yellow fever. In countries vulnerable to such epidemics, the combination of effective national laboratories and regional reference centres where further laboratory investigations can be conducted has proved to be an important and effective public health tool. These systems have enormous potential to provide a platform for the development of mechanisms to detect both emerging infections and outbreaks of disease.

Links to other health interventions

Immunization services are often widely available and potentially can support, and be supported by, additional health interventions. The combined delivery, or integration, of linked health interventions is a more effective way of achieving common health goals. For example, the benefits of combining immunization with two other interventions, namely vitamin A supplementation and the distribution of insecticide-treated nets for malaria prevention, are increasingly being seen. Such integration will require an evidence base to guide policies, strategies and investments, and methods for evaluating the impact of linked interventions. Access to integrated services needs to be systematized in order to maximize the benefits to mothers and children attending health facilities.

Preparedness for global epidemics and emergencies

Countries at risk of epidemics need preparedness plans that are firmly rooted in their overall immunization plan and services. Similarly, capacity is required at country and global levels to prepare for a rapid and appropriate response to emergencies and natural disasters since that response may involve the rational use of vaccines. In the case of influenza, a global laboratory network monitors the circulating virus strains and all countries need up-to-date preparedness plans for coping with a pandemic. Many national preparedness plans, however, do not exist, are out of date, or lack practicality. Governments, WHO, UNICEF, vaccine manufacturers and research institutes are currently involved in efforts to support the development of national preparedness plans and to expand capacity for production of influenza vaccines worldwide, including work on the development of a new vaccine against virus strains with pandemic potential.

The component strategies

Strategic Area I: Protecting more people in a changing world

Protecting more people in a changing world covers the key strategies needed to reach more people with immunization services, especially those who are hard to reach and those who are eligible for newly introduced vaccines. The aims are to ensure that every infant has at least four contacts with immunization services, to expand immunization to other age groups in an effort to maximize the impact of existing vaccines, and to improve vaccine-management systems in order to ensure immunization safety, including the availability of safe and effective vaccines at all times. The strategies in this area seek to prioritize underserved populations and areas and will use the "reaching every district" approach.

- **Strategy 1:** Use a combination of approaches to reach everybody targeted for immunization
- **Strategy 2:** Increase community demand for immunization
- **Strategy 3:** Ensure that unreached people are reached in every district at least four times a year
- **Strategy 4:** Expand vaccination beyond the traditional target group
- **Strategy 5:** Improve vaccine, immunization and injection safety
- **Strategy 6:** Improve and strengthen vaccine-management systems
- **Strategy 7:** Evaluate and strengthen national immunization programmes

Strategic Area II: Introducing new vaccines and technologies

Introducing new vaccines and technologies focuses on the need to promote the development of high-priority new vaccines and technologies and to enable countries to decide on and proceed with their introduction. The strategies in this area aim to ensure that countries have the evidence base and capacity to evaluate the need, and establish priorities, for the introduction of new vaccines and technologies, and a supply of new vaccines and technologies adequate to meet their needs, with the necessary financial resources. They also aim to ensure that new vaccines will be offered to the entire eligible population within five years of being introduced into the national programme, and that future vaccines against diseases of public health importance are researched, developed and made available, especially for disadvantaged populations with a high disease burden.

- **Strategy 8:** Strengthen country capacity to determine and set policies and priorities for new vaccines and technologies
- **Strategy 9:** Ensure effective and sustainable introduction of new vaccines and technologies
- **Strategy 10:** Promote research and development of vaccines against diseases of public health importance

Strategic Area III: Integrating immunization, other linked health interventions and surveillance in the health systems context

Integrating immunization, other linked health interventions and surveillance in the health systems context emphasizes the role of immunization in strengthening health systems through the benefits that accrue to the whole system as a result of building human resource capacity, improving logistics and securing financial resources. The aim is to link immunization with other potentially life-saving interventions in order to accelerate reduction in child mortality. The component strategies also aim to improve disease surveillance and programme monitoring so as to strengthen not only immunization programmes but the health system as a whole, and to ensure that immunization is included in emergency preparedness plans and activities for complex humanitarian emergencies.

- **Strategy 11:** Strengthen immunization programmes within the context of health systems development
- **Strategy 12:** Improve management of human resources
- **Strategy 13:** Assess and develop appropriate interventions for integration
- **Strategy 14:** Maximize the synergy from integrating interventions
- **Strategy 15:** Sustain the benefits of integrated interventions
- **Strategy 16:** Strengthen monitoring of coverage and case-based surveillance
- **Strategy 17:** Strengthen laboratory capacity through the creation of laboratory networks
- **Strategy 18:** Strengthen the management, analysis, interpretation, use and exchange of data at all levels
- **Strategy 19:** Provide access to immunization services in complex humanitarian emergencies

Strategic Area IV: Immunizing in the context of global interdependence

Immunizing in the context of global interdependence builds on the recognition that equity in access to vaccines and related financing and equal availability of information are in every country's interest. The component strategies in this area aim to increase awareness of, and respond to, the reality that every country is vulnerable to the impact of global issues and events on vaccine supply, financing, collaboration of partners, communication and epidemic preparedness.

- **Strategy 20:** Ensure reliable global supply of affordable vaccines of assured quality
- **Strategy 21:** Ensure adequate and sustainable financing of national immunization systems
- **Strategy 22:** Improve communication and dissemination of information
- **Strategy 23:** Define and recognize the roles, responsibilities and accountability of partners
- **Strategy 24:** Include vaccines in global epidemic preparedness plans and measures

Framework for planning and collaboration

The global strategy offers a broad framework rather than a detailed plan of action in order to enable all stakeholders to direct or redirect their contribution to immunization worldwide. In view of the marked differences between countries' capacities, priorities and resources, it presents a range of strategies from which countries will be able to select those most suited to their individual needs. To support this national planning process, WHO, UNICEF, multilateral and bilateral partners, nongovernmental organizations and the private sector will intensify their coordination in order to collaborate effectively with countries. The strategy urges Member States, international organizations, nongovernmental organizations, the private sector, interest groups and other stakeholders to make an unprecedented commitment to immunization at the global, national and local levels.

The way forward

The final section of the global strategy focuses on the actions needed to facilitate its implementation: consultations to ensure that countries apply the guiding principles to their own strategic planning through strategies tailored to individual needs, capacity and resources; securing the early engagement of immunization partners; concerted strengthening of the capacity of immunization services at the district level, especially in low-performing countries; establishment of a knowledge base about successfully linked health interventions as a resource for their potential scaling up; development of an evaluation and review process to measure progress up to 2015; and production and dissemination of supportive documentation detailing plans and policies, as well as further information on technical issues.

The strategic options outlined above are not exhaustive. The strategy should be seen not as a detailed blueprint but rather as an evolving plan. As the strategy and vision unfold over the next 10 years, new challenges will arise and new responses and innovations will be needed.

A vision with broad strategic directions

The global immunization vision and strategy:

- provides a vision of an expanded role for immunization in improving public health, with broad strategic directions for national policy and programme development, in the context of support to immunization programmes by all partners;
- extends the reach of immunization beyond infancy to other age groups and beyond the existing confines of immunization programmes into other settings, while maintaining the priority of vaccination in early childhood;
- encourages a package of interventions to reduce child mortality;
- contributes to global preparedness against the threat of emerging pandemics;
- commits all stakeholders to unprecedented efforts to reach the hard-to-reach;
- promotes data-driven ways of solving problems for improving programme effectiveness;
- prepares the way for the introduction and widespread use of new and underused vaccines and technologies, all of which will require long-term financial planning; and
- promotes the development of case-based surveillance for all vaccine-preventable diseases, with expansion of laboratory networks for viral and bacterial diseases.

Strategic framework for 2006–2015

Vision: A world in 2015 in which:

- *immunization is highly valued;*
- *every child, adolescent and adult has equal access to immunization as provided for in their national schedule;*
- *more people are protected against more diseases;*
- *immunization and related interventions are sustained in conditions of diverse social values, changing demographics and economies, and evolving diseases;*
- *immunization is seen as crucial for the wider strengthening of health systems and a major element of efforts to attain the Millennium Development Goals;*
- *vaccines are put to best use in improving health and security globally; and*
- *solidarity among the global community guarantees equitable access for all people to the vaccines they need.*

Guiding principles

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Ownership, partnership and responsibility
Goals are commonly agreed and pursued by governments and their partners, joined by international solidarity, which engage in coordinated activities determined by national plans.

Accountability
Stakeholders and actors in immunization are publicly accountable for their policies and actions.

Assured quality and safe products and services
All products made available meet internationally recognized standards of quality and safety, and services are delivered according to best practices.

Strong district-based immunization systems
Interventions and their monitoring at district level ensure local commitment and ownership and the appropriate adaptation of the programme to local needs and circumstances.

Sustainability through technical and financial capacity building
Financial and technical self-reliance is a target for national governments and partners working collectively, with continuing, incremental infrastructure building.

Policies and strategies based on evidence and best practices
The choice of policies, strategies and practice is informed by data from operational research, surveillance, monitoring and evaluation, disease burden and impact assessments, and economic analyses, and by the sharing of lessons and experiences from countries in similar circumstances.

Context

Introduction

Immunization is a cost-effective and life-saving intervention which prevents needless suffering through sickness, disability and death. It benefits all people, not only through improvements in health and life expectancy but also through its social and economic impact at the global, national and community level. In today's increasingly interdependent world, acting together against vaccine-preventable diseases of public health importance and preparing for the possible emergence of diseases with pandemic potential will contribute significantly to improving global health and security.

Immunization and the other linked health interventions that can be easily implemented with immunization to the benefit of both, will contribute significantly to the achievement of the Millennium Development Goals (MDGs) by improving health, especially among children and women, and contributing to poverty reduction and development efforts.[1] Specific goals that will benefit from widespread immunization include:

- reducing child mortality by two thirds between 1990 and 2015 (MDG 4), measured inter alia by using as an indicator the proportion of one-year old children immunized against measles;
- improving maternal health by reducing the maternal mortality ratio by three quarters between 1990 and 2015 (MDG 5); and
- combating HIV/AIDS, malaria and other diseases (MDG 6) by halting and beginning to reverse the incidence of malaria and other major diseases.

The Global Alliance for Vaccines and Immunization (GAVI), together with its fundraising arm The Vaccine Fund (VF), has also set milestones for selective immunization goals. These include:

- by 2010 or sooner, all countries will have routine immunization coverage at 90% nationally with at least 80% coverage in every district;
- by 2007, all countries with adequate delivery systems will have introduced hepatitis B vaccine; and
- by 2005, 50% of the poorest countries with the highest disease burden and adequate delivery systems will have introduced Hib vaccine.

A further immunization goal set by the Fifty-sixth World Health Assembly in 2003 in response to presentations on the need for global preparedness in the event of an influenza pandemic, urges Member States to increase immunization against influenza among all people at high risk, reaching 75% coverage by 2010. Countries which do not yet have a national influenza vaccination policy should establish one and draw up and implement a national preparedness plan for an influenza pandemic. Preparedness for pandemics should be further strengthened through improved national surveillance and laboratory capacity.

This document sets out a unifying vision and strategies for 2006–2015 which are intended to stimulate collective action and to ensure commitment by governments and immunization partners. It establishes a common strategic platform for countries and immunization partners, supports a comprehensive approach to planning, and urges a broader approach to immunization programmes. It is a vision that focuses not only on new vaccines and technologies for future introduction,

1. Immunization is playing an important role in reaching the goals outlined in the United Nations General Assembly Special Session for Children (2002) by reducing morbidity and mortality among all age groups. This document builds upon immunization and disease reduction goals, including those to ensure full immunization of children under one year of age at 90% nationally, with at least 80% coverage in every district or equivalent administrative unit; the global eradication of polio by 2005; the elimination of maternal and neonatal tetanus as a public health problem by 2005; the reduction of measles mortality by half between 1999 and 2005; and the extension of the benefit of new and improved vaccines and other preventive health interventions to children in all countries as appropriate.

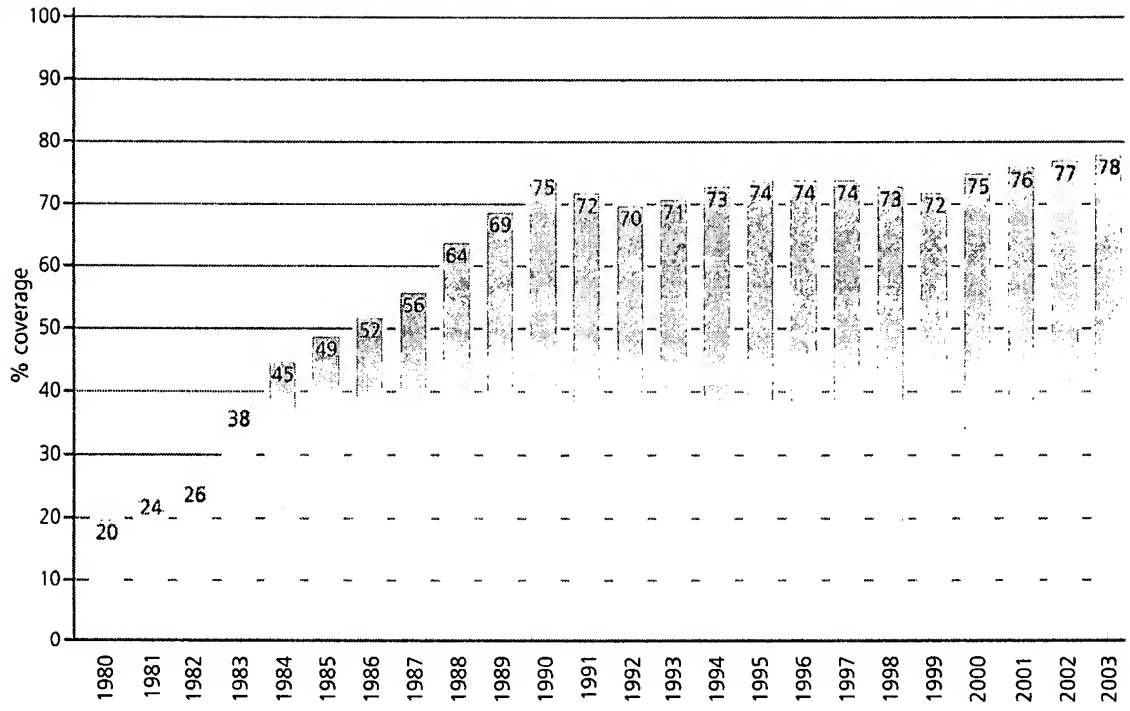
but also on ways of strengthening the current immunization system so that it can maximally deliver currently available vaccines, as well as underutilized or new vaccines and other technologies. The vision also focuses on the need to strengthen surveillance and other measurement systems in order to identify high-risk and underserved populations where additional efforts are needed, and to monitor progress. In view of the marked differences between countries' strengths and weaknesses, countries and their partners are encouraged to use this document to reflect on the policies and strategies most suited to their individual needs and circumstances.

The document offers a broad strategic framework rather than a detailed strategic plan. Building on the vision and strategic directions proposed, and informed by data available nationally and internationally, countries will be invited to examine their options, establish priorities, set objectives and targets, elaborate a national strategic plan, project their resource needs and the availability of resources, and formulate monitoring and evaluation frameworks. Towards this aim, WHO, UNICEF, multilateral and bilateral partners, nongovernmental organizations and the private sector will further strengthen their coordination in order to collaborate effectively with countries in this endeavour. To support this national planning process, the present global strategy will be underpinned by guidance documents and technical support.

History and impact

The immense scientific achievement of vaccines and immunization, targeting children and women of childbearing age in all countries, represents one of the most successful and cost-effective public health interventions in history. Immunization has eradicated small-

Figure 1: Global Immunization 1980-2003, DTP3 coverage



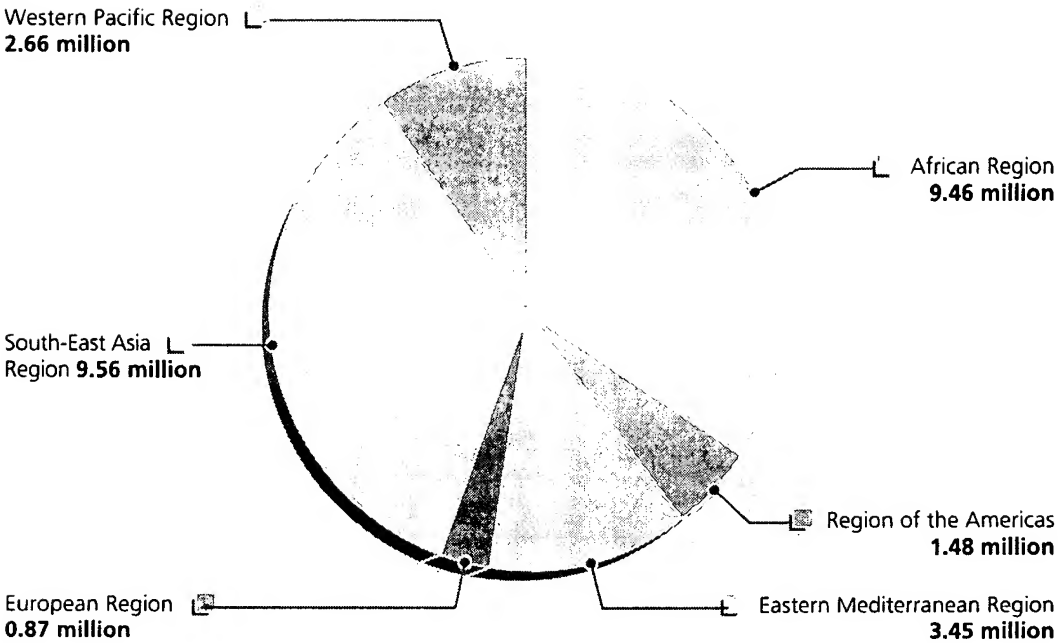
Source: WHO/UNICEF estimates, 2004

pox, substantially reduced morbidity and mortality from diphtheria, pertussis, tetanus and measles, and is on the verge of eradicating polio. Since its inception in 1974, the Expanded Programme on Immunization has provided guidance and recommendations to national authorities on how to design, develop, and manage immunization services to efficiently deliver needed immunizations. Meanwhile, during the 1980s, the global push to achieve Universal Childhood Immunization resulted in the establishment of national systems of immunization and rapidly rising immunization coverage.

Immunization services must be sustainable since over 100 million children are born every year and need to be immunized. Moreover, in an increasingly globalized world, the global community has a clear interest in the widespread use of current vaccines, as well as the rapid development of new vaccines against emerging diseases. The establishment of strong national immunization services in many countries over recent years has ensured that today over 70% of the world's targeted population is reached with immunization. As a result, it is estimated that immunization carried out in 2003 alone will prevent more than 2 million deaths from vaccine-preventable diseases and an additional 600 000 hepatitis B-related deaths (from liver cirrhosis and hepatocellular carcinoma) that would otherwise have occurred in adulthood among the children immunized in that year.

Despite these achievements, global commitment to immunization has not been sustained in all countries. In 2003 an estimated 27 million infants and 40 million pregnant women worldwide remained in need of immunization. In that same year, it is estimated that 28 million children (27% of all births) were born in 32 countries where immunization coverage is less than 70%, including 10 million in countries with coverage under 50%. In

Figure 2: 27 million children not vaccinated (DTP3), 2003[a]



Source: WHO/UNICEF estimates, 2004

[a] By WHO region.

2003, only 28% of developing countries reported that all districts had achieved over 80% coverage among infants with the basic three doses of diphtheria-tetanus-pertussis (DTP) vaccine. Moreover, beyond infancy, children, adolescents and adults do not yet fully benefit from the protection provided through immunization against diseases affecting them. For example, diseases such as measles, rubella and meningitis can cause deafness, hearing loss and other permanent disabilities which can only be prevented by immunization. These disabilities have a direct impact on efforts to meet the Millenium Development Goals on poverty reduction (MDG 1) and primary education (MDG 2).

Strength through partnerships

Global partnerships, such as GAVI/VF and the Measles Partnership, have been created in order to attain shared goals. Such partnerships bring together major stakeholders in immunization from the public and private sectors, including the vaccine industry.

Initiatives for eradication of poliomyelitis, reducing measles mortality and elimination of maternal and neonatal tetanus have shown that partnerships enable immunization services to be brought to even the most hard-to-reach communities. Through the Global Polio Eradication Initiative, for example, countries have clearly demonstrated the capacity to achieve high vaccination coverage rates and conduct high performance disease surveillance, even in areas affected by political turmoil or other difficult circumstances. However, accessing hard-to-reach populations on a regular basis and those affected by outbreaks and emergency situations requires specially designed strategies.

The Global Polio Eradication Initiative has not only achieved great progress towards its set goals but has had a wider impact on health-service delivery. It has explored new ways to engage communities in health actions benefiting them; succeeded in involving private partners and the commercial sector in immunization efforts; created high quality information systems; systematized logistics and the financial management of field programmes; and stimulated the establishment of surveillance mechanisms supported by laboratory networks. In order to build on the experience of the polio initiative, the most effective way to sustain the gains derived from polio eradication will be to gradually incorporate polio activities into disease prevention, control and surveillance, while using the valuable experience accumulated through this initiative to inform the development of future health policies and programmes.

The success of immunization depends on a sustainable and reliable supply of affordable vaccines of assured quality. Globally, 24 suppliers and more than 60 vaccines are on the WHO list of suppliers pre-qualified to supply vaccines on the international market. Although temporary shortages in global supply do occur, resulting in national stockouts, overall the outlook for the vaccine supply market is more positive than it has been for the past decade. The engagement of partnerships involving countries, international organizations and public and private industry partners will go a long way towards resolving this issue.

The polio eradication partnership has provided an excellent model that functions at every level. *Credit: WHO*

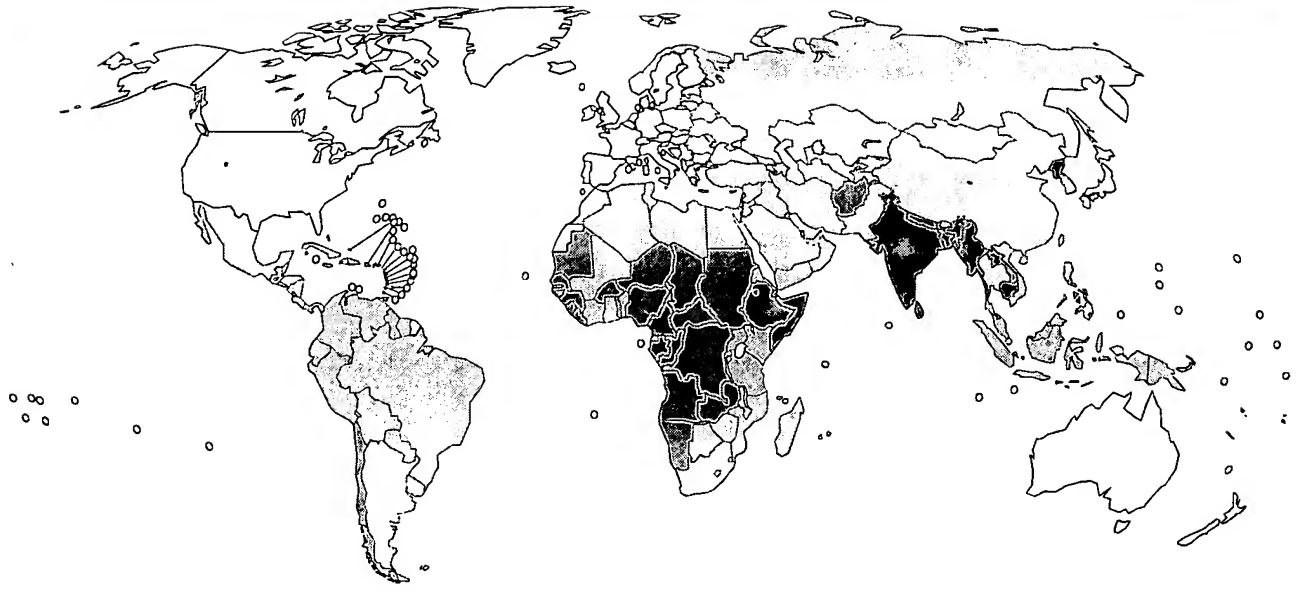
New vaccines and technologies

Efforts are under way to develop new vaccines against major infectious diseases (including malaria, HIV/AIDS and tuberculosis). Meanwhile,

many other new vaccines and technologies are already licensed or at an advanced stage of development (including rotavirus and pneumococcal vaccines), and other vaccines are readily available but underused. Activities to ensure the safety of immunization are also being implemented (such as the use of autodisable syringes) and the subject is becoming a top priority for countries.

By the end of 2003, 134 out of 165 (81%) developing countries and economies in transition had successfully introduced hepatitis B vaccine into their national immunization schedules and 63 (38%) had introduced Hib vaccine. In low-income countries, these new vaccine introductions were greatly facilitated by support from GAVI/VF.

Figure 3: 134 developing countries and economies in transition use hepatitis B vaccine in their national immunization system, as at December 2003



- 122 countries or 74% hepatitis B vaccine given to infants
- 12 countries or 7% use hepatitis B vaccine in part of the country or among adolescents
- 31 countries or 19% do not use hepatitis B vaccine

Source: WHO/UNICEF Joint Reporting Form, 2004. Data collected from 192 WHO Member States, as at 20 September 2004.

During the period 2006–2015, countries may be faced with an unprecedented array of new vaccines and technologies for introduction. To ensure that countries can make rational, evidence-based decisions about the choice of new vaccines and technologies, current gaps in knowledge (including disease burden, the cost-effectiveness of various strategies, and regulatory issues) will have to be filled.

Financing

In low-income countries, especially in sub-Saharan Africa, overall health services are desperately under-financed. In some countries, basic health services receive less than US$ 10 a year per capita – against a requirement of US$ 30–40 a year per capita. Although immunization

financing should be primarily a national public responsibility, many low-income countries rely heavily on international assistance for this. As a result, financing can be volatile and vulnerable to shifts in donor priorities. In response, a number of innovative financing mechanisms have been developed, including the Pan-American Health Organization's Revolving Fund for Vaccine Procurement and the Vaccine Independence Initiative, GAVI/VF, as well as the inclusion of immunization in country Poverty Reduction Strategic Papers and Sector-Wide Approaches.

Immunization is a highly cost-effective and relatively inexpensive health intervention. The overall cost of immunization, however, including the procurement of new vaccines, new vaccine formulations and technologies, is expected to rise sharply in the future. The expansion of vaccination schedules to include new vaccines has greatly increased the amount of resources that need to be mobilized. Although some relief may be obtained over time as the larger amounts of vaccine to be procured leads to greater competition among manufacturers and a reduction in price, experience has shown that it takes several years before increased demand for new vaccines is matched by lower prices. Meanwhile, the rising cost of immunization delivery needs to be added to the cost of vaccines; logistics and labour are becoming more expensive, and the extension of services to populations that are currently not being reached will need additional resources.

Securing the financing for the introduction of new vaccines and increasing coverage with existing vaccines will test all countries and their partners. Ways need to be found to maximize the cost-effectiveness of contacts with immunization services (such as spreading the cost of these contacts across relevant health initiatives) and to strengthen national capability to project financial needs and obtain the required resources.

Evidence-based policy decisions will have to be taken on the "affordability" of vaccines in relation to the reduction of disease burden. In view of this, it will be critical to reinforce the benefits of vaccines and immunization and to highlight their importance as a highly valued component of primary health care. In both relative and absolute terms, governments and immunization partners may have underinvested in immunization, partly due to expectations that the cost of vaccines would always remain no more than a few cents per dose. Over recent years, traditional vaccines, whilst remaining relatively inexpensive, have for a variety of reasons increased in price. New vaccines will be even more expensive, while continuing to provide an excellent public health intervention.

It is anticipated that, in the near future, additional broad-based funding mechanisms such as the front-loading of immunization support through international financing facilities may enable further expansion of support through GAVI/VF or other channels. Meanwhile, the returns from investments made in different health sector areas will be maximized through the proposed linkage of other disease-targeted interventions with immunization, to the benefit of both.

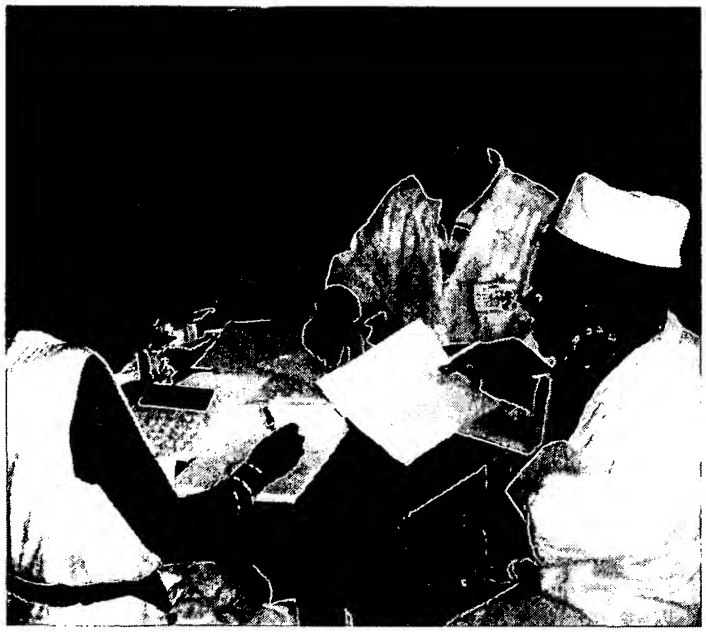
Contribution to overcoming system-wide barriers

Increasingly, immunization will help to overcome barriers to equitable health-service delivery and sector-wide development, and will benefit from those efforts. The benefits include better public health and improved efficiency of public health services. Immunization services inevitably experience the constraints that affect the health system as a whole, but they can help significantly in overcoming system-wide barriers through the strengthening of district teams and their capacity to make optimal use of the resources and opportunities available locally. In turn, sector-wide approaches to strengthening cross-cutting areas such as human resources management, financing, logistics, public–private partnerships and information sharing can clearly benefit immunization.

For example, immunization relies on a sector-wide approach to obtain a share of national and international funding sources as well as national accounting and financing systems. At the same time, immunization can also contribute experience in fund-raising as well as the capacity to engage local donors. Similarly, while immunization benefits from a sector-wide approach to medium-term and long-term planning and financing of logistics, it can contribute considerable experience in managing transportation, health-care waste disposal and the cold-chain system, as well as having an overall structure and systems in place. The inclusion of immunization services as a key component of health-system development will greatly enhance efforts to achieve greater integration of services and long-term financial sustainability.

Strong monitoring, surveillance and evaluation capacity

Over the past decade, considerable progress has been made in establishing systems for monitoring and surveillance of coverage rates and trends of vaccination and its impact on vaccine-preventable diseases, and in using those data for guiding public policy, strategies and programmes. The extensive and growing laboratory networks for poliomyelitis and measles have not only generated crucial information for guiding the respective eradication and mortality reduction initiatives, but have also supported the prevention and control of epidemics of, for instance, meningitis, diphtheria, rubella and vector-borne diseases such as dengue and yellow fever. In countries vulnerable to such epidemics, the combination of effective national laboratories and regional reference centres where further laboratory investigations can be conducted has proved to be an important and effective public health tool. These systems have enormous potential to provide a platform for the development of mechanisms to detect both emerging infections and outbreaks of disease. With the expanded use of underutilized vaccines (e.g., Hib, rubella, yellow fever) and the targeting of additional diseases over the coming decade (e.g., rotavirus, pneumococcal disease and meningococcal disease), case-based surveillance for all vaccine-preventable diseases and, more generally, monitoring and evaluation of immunization systems will enhance national and global ability to adapt strategies to evolving needs. Such information will help determine their cost-efficiency and cost-effectiveness, strengthen accountability at all levels and, together with the Global Alert and Response Network for emerging diseases and epidemics, contribute to global health and security.



District team compiles a monthly surveillance report.
Credit: WHO

Links to other interventions

Immunization services are often widely available and potentially can support, and be supported by, additional health interventions. The combined delivery, or integration, of linked health interventions is a more effective way of achieving common health goals. For example, the benefits of combining immunization with two other interventions, namely vitamin A supplementation and the distribution of

insecticide-treated nets for malaria prevention, are increasingly being seen. Integration may also involve combining preventive and care services – offering timely immunization and treatment through a single delivery channel. In some cases, disease-targeted activities have the potential to expand the benefits of immunization contacts. At fixed health facilities, immunization is often combined with other services such as growth monitoring, nutritional advice, information on preventive care, referral of the child for medical conditions, and reproductive and sexual health care for the mother. However, efforts are needed to ensure that these interventions are also integrated into outreach immunization activities.

For those who are responsible for national planning, integration means bringing together the management and support functions of different sub-programmes, and ensuring the complementarity between different levels of care. The integration of immunization services with other health interventions will require an evidence base to guide policies, strategies and investments, as well as a means to evaluate the impact of linked interventions. Access to integrated services needs to be systematized in order to maximize the benefits to mothers and children attending health facilities.

Preparedness for global epidemics and emergencies

Some vaccine-preventable diseases occur periodically in the form of widespread regional epidemics, only to fade away for several years before reoccurring. Examples of these epidemic diseases include meningococcal meningitis, yellow fever and Japanese encephalitis. Countries at risk of epidemics need preparedness plans that are firmly rooted in their overall immunization plan and services. Similarly, capacity is required at country and global levels to prepare for a rapid and appropriate response to emergencies and natural disasters since that response may involve the rational use of vaccines. In the case of influenza, a global laboratory network monitors the circulating virus strains and all countries need up-to-date preparedness plans for coping with a pandemic. Many national preparedness plans, however, do not exist, are out of date, or lack practicality. Governments, WHO, UNICEF, vaccine manufacturers and research institutes are currently involved in efforts to support the development of national preparedness plans and to expand capacity for production of influenza vaccine worldwide, including work on the development of a new vaccine against virus strains with pandemic potential.

Responding to needs: the global strategy

Immunization services throughout the world have achieved remarkable progress to date, mainly through a focus on infants and with only a limited number of vaccines available to developing countries. However, over the past two decades coverage levels have stagnated at sub-optimal levels in many countries. Globally, out of every four children born each year, one will not receive commonly available vaccines and, as a result, will be exposed to morbidity, disability, stunted growth or premature death that could have been averted by timely immunization.

The global strategy proposes to *sustain* immunization to those who are currently reached, *extend* immunization to those who are currently unreached and to age groups beyond infancy, *introduce* new vaccines and technologies and *link* immunization to other health interventions as well as to the development of the overall health system. It places immunization firmly within the context of sector-wide approaches to health, highlighting the way immunization can both benefit from and contribute to health-system development and the alleviation of system-wide barriers.

This document offers a framework within which national policies, programmes and action plans can be elaborated. The global strategy takes on the challenges of increasing access, the target population, and the range of products that will become available. It outlines what needs to be done at global, country and service-delivery level, in a way that can be adapted for regional and national strategic plans. It offers the potential to use available budgets more efficiently and to ensure better coordination between all stakeholders towards the reduction of vaccine-preventable diseases, disability and deaths.

Vitamin A capsules and polio vaccine are often simultaneously administered during polio vaccination campaigns.
Credit: WHO

Goals

Between 2006 and 2015, all those working on immunization and related product development should strive to prevent morbidity and mortality by achieving the following goals and targets.

By 2010 or earlier

- **Increase coverage.** Countries will reach at least 90% national vaccination coverage[2] and at least 80% vaccination coverage in every district or equivalent administrative unit.
- **Reduce measles mortality.** Globally, mortality due to measles will have been reduced by 90% compared to the 2000 level.

By 2015 or earlier *(as the case may be)*

- **Sustain coverage.** The vaccination coverage goal reached in 2010 will have been sustained.
- **Reduce morbidity and mortality.** Global childhood morbidity and mortality due to vaccine-preventable diseases will have been reduced by at least two thirds[3] compared to 2000 levels.
- **Ensure access to vaccines of assured quality.** Every person eligible for immunization included in national programmes will have been offered vaccination with vaccines of assured quality according to established national schedules.
- **Introduce new vaccines.** Immunization with newly introduced vaccines will have been offered to the entire eligible population within five years of the introduction of these new vaccines in national programmes.
- **Ensure capacity for surveillance and monitoring.** All countries will have developed the capacity at all levels to conduct case-based surveillance of vaccine-preventable diseases, supported by laboratory confirmation where necessary, in order to measure vaccine coverage accurately and use these data appropriately.
- **Strengthen systems.** All national immunization plans will have been formulated as an integral component of sector-wide plans for human resources, financing and logistics.
- **Assure sustainability.** All national immunization plans will have been formulated, costed and implemented so as to ensure that human resources, funding and supplies are adequate.

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2. Referring to vaccines containing all antigens given to children under one year of age, those containing measles antigen for children up to two years of age, and those given to women of childbearing age, as provided for in national immunization schedules. In the case of newly introduced vaccines, these should have been introduced in a country's national schedule for at least five years.

3. This goal correlates with Goal 4 of the Millennium Development Goals with its target of reducing the under-five mortality rate by two thirds between 1990 and 2015. It is expected that the additional reduction in mortality will be achieved through effective case management. Assuming a rapid increase in access to vaccines, including the introduction of new vaccines and the greater use of underused vaccines, it is expected that the two thirds reduction in mortality due to vaccine-preventable diseases will be mainly achieved through a 70–80% reduction in the number of deaths from diseases that are currently vaccine-preventable (i.e. measles, pertussis, diphtheria, tetanus and illness due to *Haemophilus influenzae* type b infection) once coverage reaches 90%, and a 40–50% reduction in deaths from diseases that are expected to be prevented by new vaccines in the near future (i.e. against rotavirus and pneumococcal infection). This estimate will be revised over time, as better projections are developed and better data become available.

Realizing the vision

The global strategy comprises four main areas with 24 component strategies. The strategic approaches are: protecting more people in a changing world; introducing new vaccines and technologies; integrating immunization, other linked health interventions and surveillance in the health systems context; and immunizing in the context of global interdependence.

Strategic Area I

Protecting more people in a changing world covers the key strategies needed to reach more people with immunization services, especially those who are hard to reach and those who are eligible for newly introduced vaccines.

Strategic Area II

Introducing new vaccines and technologies focuses on the need to promote the development of high priority new vaccines and technologies and to enable countries to decide on and proceed with their introduction.

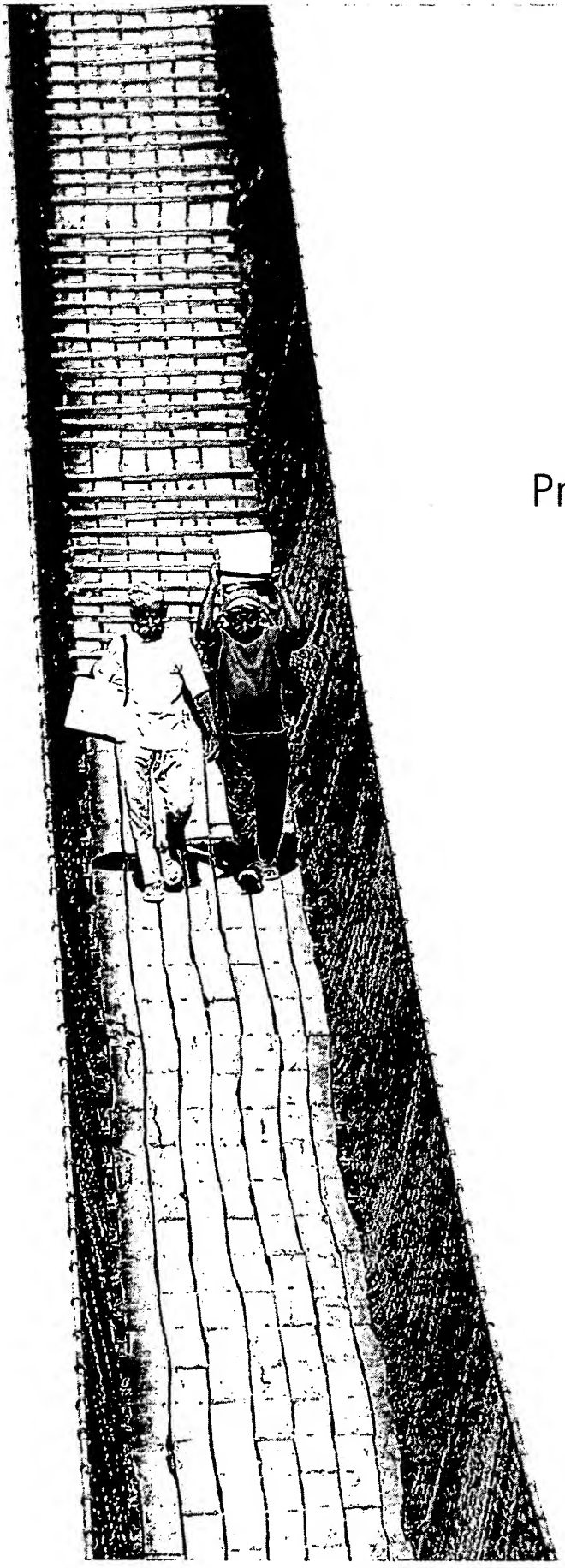
Strategic Area III

Integrating immunization, other linked health interventions and surveillance in the health systems context emphasizes the role of immunization in strengthening health systems through the benefits that accrue to the whole system as a result of building human resource capacity, improving logistics and securing financial resources. The aim is to link immunization with other potentially life-saving interventions in order to accelerate reduction in child mortality. The component strategies also aim to improve disease surveillance and programme monitoring so as to strengthen not only immunization programmes but the health system as a whole, and to ensure that immunization is included in emergency preparedness plans and activities for complex humanitarian emergencies.

Strategic Area IV

Immunizing in the context of global interdependence builds on the recognition that equity in access to vaccines and related financing and equal availability of information are in every country's interest. The component strategies in this area aim to increase awareness of, and respond to, the reality that every country is vulnerable to the impact of global issues and events on vaccine supply, financing, collaboration of partners, communication and epidemic preparedness.

Each strategic area is characterized by a brief statement on current knowledge, experience and key challenges. This is followed by an outline of the specific aims, strategies and key actions that may be considered in developing national strategic plans. In this way, the proposed strategies create a framework for national, institutional and local planning and budgeting. In addition, they are intended to provide a framework in which partner support can operate. The focus is on what needs to be done and how this may be achieved, recognizing that the final choice of strategies will be made by individual countries, based on their capacity, priorities and the projected availability of resources.



Strategic Area I

Protecting more people in a changing world

Challenges

Most of the children currently unreached by immunization live in the least-developed countries, which carry a disproportionate share of the world's disease burden. However, there are unreached populations and immunization system failures in every country. Although immunization services have been strengthened in many countries and succeed in averting many deaths, there is continuing concern at the failure to achieve high immunization coverage in every district. At the global level, and in some regions, immunization coverage (measured by coverage among infants with three doses of DTP) has stagnated since the early 1990s. Although progress has been achieved since then, in 2003 coverage remained below 50% in 12 countries, all in Africa. In 2003, over 27 million children worldwide were not immunized during their first year of life and will remain vulnerable to vaccine-preventable diseases unless comprehensive multi-year national and district plans are developed, implemented and monitored. Both national and district level planning will have to prioritize not only underserved populations and areas, but also accessible but unreached urban and periurban populations, and develop specific strategies to reach them.

Valuable lessons have been learned which can be applied to strengthen immunization services. Experience from disease-control activities for polio, measles, neonatal tetanus, yellow fever and epidemic meningitis demonstrates that, when appropriate policies, programmes and resources are in place, children and women can be reached with immunization even in the most difficult and remote areas and where routine health services are not readily available.

However, in many countries, insufficient and inadequately planned financial resources and poor budgetary and financial management compromise the sustainability and expansion of health and immunization services. Poor management and economic crises have led to funding deficits, resulting in inadequate resources at local levels for supervision, training of staff, and logistic support, as well as the cancellation of outreach services in some areas.

In many countries, underserved populations remain unreached due to poor planning, ineffective delivery strategies or weak implementation. All too often countries lack national and district level immunization workplans, and the district-level management that exists is often over-burdened, untrained or weak. Poor management in some countries is evidenced by high drop-out rates and by missed opportunities for immunization. Immunization schedules in many countries do not cover age groups beyond infancy. Elsewhere, countries often do not have effective strategies in place to reach these other age groups (e.g., school-aged children, adolescents and adults). As a result, the full potential of available vaccines is not being realized.

Countries also need to ensure that they have efficient vaccine-management systems and logistics. Vaccine quality must be maintained at every

Reliable immunization sessions are the key to protecting more children. *Credit: WHO*

stage of the continuum between manufacture and administration of the vaccine. The storage and transportation of vaccine after arrival in a country are the weak links in the supply and delivery chain. Meanwhile, the availability of heat-stable vaccines, which are often sensitive to freezing, brings new challenges as well as more cost-effective approaches that can reduce dependency on the traditional cold chain.

Immunization safety is another critical issue for immunization programmes. As a preventive intervention, immunization is wholly reliant on the acceptance, understanding and trust of those who use the services. Immunization safety (including vaccine quality and safety, injection safety and safe injection waste disposal) is therefore a critical component of the trust placed by clients in immunization services.

Meanwhile, the promotion of immunization as a key public health strategy is currently not utilized to its full capacity. As a result, many people remain unaware of the risks of vaccine-preventable diseases and/or the benefits of immunization, and immunization has become increasingly undervalued.

Routine immunization services need to reach people living in hard-to-reach areas. *Credit: WHO*

Aims

- Achieve a minimum of four immunization contacts with all infants, especially among hard-to-reach populations (geographically, socially or culturally), using a district-based approach that provides immunization through fixed sites, outreach services, mobile teams, supplementary immunization and the private sector.
- Expand immunization beyond infancy to other age groups to maximize the impact of existing vaccines.
- Use appropriate strategies to reach at-risk populations in order to rapidly reduce disease burden and to prevent and respond to epidemics and outbreaks.
- Strengthen vaccine-management practices, including logistics, to ensure the availability of safe and effective vaccines at all times.

The component strategies

Strategy 1: Use a combination of approaches to reach everybody targeted for immunization

Efforts to ensure that everyone is reached with immunization services will require the political and financial commitment of all stakeholders. Immunization can be delivered through routine services and/or supplementary activities. Most countries – both industrialized and developing – use a combination of these approaches in order to respond appropriately to changing disease patterns or to rapidly increase herd immunity through high immunization coverage. A balanced approach which combines routine and supplementary immunization will avoid any potential diversion of attention and resources. By methodically building on the experience gained from polio eradication activities, maternal and neonatal tetanus elimination and measles control/elimination, and ensuring coordinated and comprehensive planning and resource availability, countries will be able to avoid the fragmentation of immunization activities and ensure sustained high coverage with current and new vaccines.

In countries or districts where existing strategies have produced high vaccination coverage and good access to health services, care should be taken to sustain these gains by maintaining the human, financial and programmatic support. In all cases, pursuing the "Reaching Every District" (RED) approach described in Strategy 3, will be a key factor in efforts to maintain the coverage reached.

Strategy 1: Activities

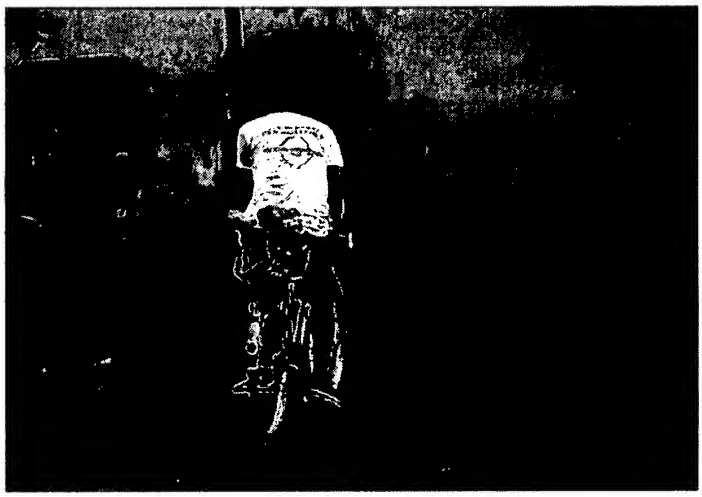
- *Strengthen national commitment to ongoing immunization services through policy and strategy development that also includes human resources and financial planning with national budget allocations, in the context of a wider health sector strategic plan.*

  Formulate and implement comprehensive multi-year national strategic plans and annual workplans to deliver reliable services based on data analysis and problem solving. The plans and budgets should cover all areas supporting the national immunization programme, including routine vaccination, accelerated disease-control activities, introduction of new vaccines, surveillance, laboratory support, financing logistics, vaccine management, cold-chain management, social mobilization and communication.

  Sustain high vaccination coverage where it has been achieved, by ensuring that adequate support is maintained for existing systems.

- *Develop appropriate national strategies to immunize children who were not immunized during infancy.*

  Where and when appropriate, include supplementary immunization activities as an integral part of the national plans to achieve national goals.



In some places 50% of the country can only be accessed through outreach. *Credit: WHO*

Strategy 2: Increase community demand for immunization

Coverage increases if there is community demand and well-informed confidence in the benefits and safety of immunization and in the need to adhere to a prescribed schedule. The availability of adequate means of communicating these benefits to the public and the existence of responsive and reliable health services delivering an integrated health-care package will stimulate the demand needed to drive the programme forward.

Communication at the village level is a vital strategy for immunization. *Credit: WHO*

Strategy 2: Activities

- *Engage community members, nongovernmental organizations and interest groups in immunization advocacy and implementation.*
- *Assess the existing communication gaps in reaching all communities and develop and implement a communication and social mobilization plan as part of the comprehensive multi-year plan based on these assessments. The plan should include ways of targeting unreached communities, establishing well-informed community demand, and addressing the problem of immunization refusal.*
- *Provide regular, reliable, and safe immunization services that match demand.*

Strategy 3: Ensure that unreached people are reached in every district at least four times a year

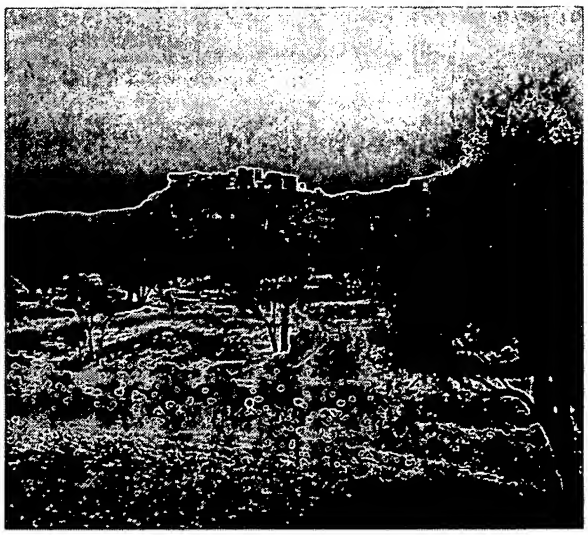
Under normal circumstances, it is possible to fully immunize children with at least four contacts with immunization services during their first year of life. However, some children remain unreached for reasons of geographic isolation, lack of information, the social or cultural environments in which they live, or active discrimination to which they are subjected. Therefore, special measures are needed to overcome all these barriers. Reaching unreached urban populations will become an important component of this strategy as urbanization and informal settlements place urban children increasingly at risk of missing out on immunization.

Five operational components needed for "Reaching Every District" (RED):

- *Re-establishment of regular outreach services*
- *Supportive supervision: on-site training*
- *Community links with service delivery*
- *Monitoring and use of data for action*
- *Better planning and management of human and financial resources.*

Strengthened efforts and additional resources may be required to establish or expand outreach services through a combination of short-term and longer-term approaches (e.g. mobile services combined with the creation or revitalization of permanent services). Underserved populations and areas will be prioritized and, through the use of the RED approach, efforts will be directed to achieving greater equity in the availability and delivery of immunization services. The design and maintenance of strong outreach services with the capacity for the successful introduction of new policies and interventions are dependent on specific knowledge and skills among immunization providers, as well as regular supportive supervision. To meet this need, integrated and regular district-based training, supervision and follow-up, together with the creation and periodic assessment of supervision standards, targets and plans will aim to ensure the timeliness and quality of services. In addition, it is important that financial resources, logistic support and supplies should be regularly available to ensure that district teams are able to fulfil their role.

Mobile vaccination teams travel over rough terrain and long distances to reach remote settlements. *Credit: UNICEF*



District plans need to include every community in the area. *Credit: WHO*

Strategy 3: Activities

- *Through microplanning at the district or local level, map (geographically, socially, culturally) the entire population in order to identify and reach the unreached target populations at least four times a year.*
- *Reduce the number of immunization drop-outs (incomplete vaccination) through improved management, defaulter tracing, and social mobilization and communication during immunization contacts, and avoid missed opportunities to vaccinate.*
- *Strengthen the managerial skills of national and district immunization providers and managers and develop and update supervisory mechanisms and tools.*
- *Provide timely funding, logistic support and supplies for programme implementation in every district.*

Strategy 4: Expand vaccination beyond the traditional target group

Expanding the benefits of immunization to population groups other than infants and women of childbearing age – for example, to older children for booster doses and to adults for epidemic prevention and control – has the potential to prevent even more morbidity and mortality and to increase global security against impending pandemics. In developing countries today, routine immunization services are only offered to infants and women of childbearing age. As a result, children and women who are not immunized on schedule remain susceptible to vaccine-preventable diseases and may benefit from vaccines only when supplementary vaccination activities are carried out (against measles or polio, for example). However, in the intervening period, those who remain unimmunized may not only acquire but also spread such diseases.

In addition, booster doses may be required in order to consolidate immunity against diseases such as diphtheria, tetanus and pertussis, while a second opportunity to receive measles vaccine may imply reaching out routinely to children older than one year. Other vaccines (for example, against influenza) and interventions (for example, vitamin A and iron supplementation) are of direct benefit to children and adults and, in particular, to those who for reasons of age, ongoing illness or disability are at greater risk than others of acquiring severe forms of infection.

The expansion of immunization services beyond the current age groups is no small task. It requires efforts to gather and analyse the information needed to support policies and investments, as well as the possible reorientation or creation of services targeting these new populations. The benefits of such efforts are twofold: the reduction of preventable morbidity and mortality in individuals who have been immunized; and the establishment of systems that can be used in the event of emerging epidemics affecting children and adults alike.

A woman receiving tetanus toxoid (TT) immunization during TT supplemental immunization activities in rural Yemen.
Credit: UNICEF

Strategy 4: Activities

- *As part of national policy and strategy development, define target populations and age groups for vaccination appropriate to the national situation, making the protection of those outside the infant age group an integral component of immunization services.*
- *Apply standard tools to assess the cost-effectiveness of different immunization schedules and strategies in a range of demographic, geographic and epidemiological settings.*

Strategy 5: Improve vaccine, immunization and injection safety

Safe immunization requires safe and potent vaccines, safe injection practices and adequate sharps waste disposal, and rapid action when adverse events occur following immunization. Immunization safety provides a model and sets standards of practice applicable to the wider spectrum of injections performed in the health-care setting.

Health workers are trained to ensure they deliver immunizations safely. *Credit: WHO*

Strategy 5: Activities

- *Procure vaccines only from sources that meet internationally recognized quality standards.*
- *Ensure long-term forecasting for existing and new vaccines by improving vaccine-management skills.*
- *Achieve national self-reliance in quality assurance and regulatory oversight to meet global standards, and promote and further strengthen existing programmes that support this (in particular, scientific evaluation, capacity building, public education, training and communication).*
- *Introduce, sustain and monitor safe injection practices, including the use of autodisable syringes and other safe methods of vaccine administration, and thereby contribute to the enforcement of safe injection practices and health-care waste disposal.*
- *Establish surveillance and response to adverse events following immunization, both for existing vaccines and for new vaccines as they are introduced into national schedules.*
- *Be responsive to potential vaccine safety issues and address these urgently.*

Strategy 6: Improve and strengthen vaccine-management systems

One of the main challenges that immunization programmes face today is the need to ensure that vaccines are not damaged due to mishandling after their arrival in the country. The transport and storage of vaccines under controlled temperatures is important to ensure their safety and potency. At the same time, vaccines must reach all those they are intended for, including those living in remote and underserved areas. Poor vaccine stock management at all levels, poor vaccine handling and storage and high wastage contribute to the poor performance observed in immunization programmes. In many countries, the continuing disintegration of the cold chain and vaccine distribution mechanisms has prompted efforts to improve vaccine-management systems and to strengthen the immunization programme infrastructure.

An additional challenge is the need to ensure adequate supplies of vaccine and equipment nationwide without disruption. Inefficient vaccine-management systems – including poor stock management, poor quality of vaccine handling and storage and high wastage – contribute substantially to low programme performance. Between 2006 and 2015, cold-chain equipment will need to be replaced at least once. Meanwhile, the introduction of new vaccines will require new strategies and additional cold storage capacity.

Recent developments such as the improved heat stability of vaccines, increased awareness of the need to prevent freezing, and the availability of vaccine vial monitors on most vaccines supplied through UNICEF have opened the way for innovative approaches in vaccine management which have the potential to reduce dependency on the traditional cold chain.

The logistics of immunization also require adequate and well-functioning transportation and communication systems, currently often deployed solely for immunization programmes. This investment should increasingly be considered in the context of an expanded package of linked health interventions, allowing for cost sharing, joint training and effective management.

Strategy 6: Activities

- *Conduct accurate demand forecasting at national and district levels to ensure the uninterrupted supply of assured quality vaccines, autodisable syringes and safety boxes, and new types of equipment as they become available. Forecasting should be reviewed regularly to respond to changing delivery strategies.*
- *Build capacity for effective vaccine management through training, supervision and the development of information systems in order to ensure the safety and potency of vaccines up to the point of use.*
- *Increase access and coverage through a "safe chain" approach which includes taking vaccines beyond the cold chain, using a vaccine vial monitor-based system for vaccine-management.*
- *Move towards coordinated and sector-wide financing and management for transportation and communications.*

Strategy 7: Evaluate and strengthen national immunization programmes

The performance of immunization activities will be guided by disease surveillance, coverage monitoring and management information. These are addressed in Strategic Area III in view of their strong linkages to system-wide disease and management monitoring processes. In addition, the design, reorientation, further expansion and validation of immunization strategies will be informed by focused immunization programme evaluations and operations research. Information arising from programme reviews and other forms of evaluation will be shared widely in a spirit of transparency and accountability towards all involved.

Strategy 7: Activities

- *Conduct regular immunization programme evaluations at local, district and national levels and provide feedback on performance, obstacles and new opportunities to all partners.*
- *Where appropriate, perform operations research and evaluation of "what works" to improve the delivery of immunization and to make systems more effective, efficient and equitable in order to improve immunization coverage.*

Strategic Area II
Introducing new vaccines and technologies

Challenges

Many major breakthroughs are occurring in the development of new vaccines and technologies. Through the support of GAVI/VF, major progress has been made in making new vaccines available in low-income countries. These advances are revolutionizing the way vaccines are conceptualized, manufactured, presented and administered. It is critical that the setting of research priorities and financial investments take into account the needs of both developed and developing countries and, within these countries, of populations that are the most vulnerable to ill health and premature death.

Combination vaccines that include DTP with other antigens (e.g., hepatitis B and Hib) offer opportunities to simplify immunization delivery and are expected to become increasingly available during the next decade. However, the availability of different combination vaccines will pose new challenges for immunization programmes. Difficult choices will have to be made not only between monovalent vaccines and more expensive combination vaccines, but also between the different combination vaccines available.

While existing vaccines (such as Hib, yellow fever, influenza, Japanese encephalitis and rubella vaccines) are readily available but underused, new vaccines against rotavirus, pneumococcus, meningococcus, and human papilloma virus are in advanced stages of testing or already being introduced on a limited scale. At the same time, new vaccines are being developed against major infectious diseases such as malaria, HIV/AIDS, pandemic influenza and tuberculosis, as well as against some of the "orphan" infectious diseases, including leishmaniasis and hookworm infestation.

In addition, various new immunization-linked methods and products are being developed to increase the ease, safety and efficacy of immunization delivery. It is anticipated that several of these – for example, new formulation methods to increase vaccine stability, devices for the non-injectable administration of vaccines, and rapid biomedical tests to monitor and evaluate the impact of immunization – will be helpful in accomplishing the goals set forth in this document. Once tested, these new methods and devices will have to be brought to the market at affordable prices if they are to be used widely in low-income countries.

New vaccines are being developed against major infectious diseases such as malaria, HIV/AIDS, pandemic influenza and tuberculosis. *Credit: WHO*

Decisions on the introduction of new or underutilized vaccines and new technologies must be based on country disease burden and priorities and on what is affordable and sustainable within the budgeting and planning context. Countries should be empowered to evaluate their own needs and priorities for new and underutilized vaccines and technologies. Critical issues will include how new vaccines can help immunization systems reach more people and stimulate community, country and partner commitment to sustaining access to these products in the long term at affordable costs. The introduction of new vaccines will also require countries to reassess their immunization schedules, including the need for booster doses, immunization at school entry, and the immunization of adolescents and adults.

Partners, according to their mandates, will continue to fulfil their roles towards regulating and assuring the quality and availability of these products through the strengthening of regulatory and delivery systems. For countries where the introduction of new vaccines will be co-financed by external sources, this must involve collaboration between governmental and nongovernmental entities, international development and technical agencies, other interest groups, and the private sector. The role of partner coordinating bodies such as the Interagency Coordinating Committees will be critical in efforts to coordinate partner inputs as well as to ensure the long-term operational and financial sustainability of immunization services after the introduction of new products. The early involvement of ministries of finance and of the national political leadership will be necessary to ascertain that the availability of financial resources will match the planned commitments in the long term.

Finally, despite the momentum to develop new vaccines and formulations, there are also disincentives to the development of vaccines perceived as low-profit products for developing country markets. As a result, research agendas are often tailored to the needs of wealthier countries instead. To overcome these barriers will require new approaches to stimulate and support research, development and regulatory processes across the world, together with an increased focus on capacity building in developing countries and the development of public and private financing mechanisms that create incentives for technical cooperation, other forms of partnership and fair competition.

Current and future vaccines and technologies

Current vaccines

- BCG [a]
- Cholera (inactivated and live) [b]
- DTP and DTP-based combinations [a]
- *Haemophilus influenzae* type b [a]
- Hepatitis A [a]
- Hepatitis B [a]
- Influenza [a]
- Japanese encephalitis (inactivated and live) [b]
- Measles [a]
- Meningococcus (polysaccharide and conjugate) [a]
- Mumps [a]
- Pneumococcus (polysaccharide and conjugate) [a]
- Polio (OPV and IPV) [a]
- Pseudomonas [b]
- Rabies [b]
- Rift Valley fever [b]
- Rubella [a]
- Tetanus toxoid [a]
- Tick-borne encephalitis [b]
- Typhoid [b]
- Varicella [a]
- Yellow fever [a]

Available but underused immunization supportive technologies

- Pre-filled injection devices
- Vaccine vial monitors on all vaccines

[a] *Available for immediate use in routine immunization.*
[b] *Available for specific regions or circumstances.*

New or improved vaccines anticipated by 2015

- Dengue [d]
- DTaP (with two P antigens) [d]
- Enterotoxigenic *Escherichia coli* (ETEC) [d]
- Group A streptococcus [d]
- Human papilloma virus [c]
- Influenza for pandemic response
- Japanese encephalitis (improved) [c]
- Malaria [d]
- Measles (aerosol) [c]
- Meningococcus A (multi-serotype conjugate) [c]
- New combinations of existing vaccines [d]
- Pneumococcus (improved conjugate or protein-based) [c]
- Polio (inactivated vaccines based on Sabin strains) [c]
- Polio (monovalent OPV type 1) [d]
- Respiratory syncytial virus [d]
- Rotavirus [c]
- Severe acute respiratory syndrome (SARS) [d]
- Shigella [d]
- Typhoid (conjugate) [d]
- West Nile fever [d]

New immunization supportive technologies anticipated by 2015

- Jet injectors
- Thermostable vaccines
- Vaccine aerosols
- Vaccine nasal sprays
- Vaccine patches

[c] *In a late stage of development.*
[d] *Licensing expected in 2010–2015.*

Aims

- Empower countries to evaluate the need and establish priorities for introducing newly available vaccines and technologies through strengthening their capacity to make decisions based on disease burden, economic analysis and the feasibility of introduction.
- Ensure that countries have an adequate supply of new vaccines and technologies to meet their needs and have access to the corresponding financial resources.
- Ensure that new vaccines will be offered to the entire eligible population within five years of the introduction of these new vaccines in national programmes.
- Ensure that future vaccines of public health importance are researched, developed and made available, particularly for disadvantaged populations with a high disease burden.

The component strategies

■ Strategy 8: Strengthen country capacity to determine and set policies and priorities for new vaccines and technologies

The possible addition of a new vaccine or new technology to an immunization delivery system requires careful consideration of disease burden in relation to other public health priorities and programmatic feasibility. In addition, mechanisms to ensure the long-term financial sustainability of the new vaccine or technology need to be in place prior to introduction. The promising development of several new vaccines makes it even more important to help countries determine which of these represents the best opportunity for the investment of limited national resources, in particular which product will be easiest to integrate into immunization systems and has the potential to provide the greatest public health benefit.

Strategy 8: Activities

- *Strengthen country capacity to assess disease burden and the cost and cost-effectiveness of new vaccines and technologies through the use of standard tools.*
- *Characterize the optimal product formulations and schedules to maximize impact and minimize cost and operational difficulties.*
- *Assist the country decision-making process, build an evidence base of country experience and methodology at the international level for each new vaccine and technology.*
- *Ensure that the long-term financial requirements from national governments and supporting partners are fully understood and committed to prior to the introduction of new vaccines.*

■ Strategy 9: Ensure effective and sustainable introduction of new vaccines and technologies

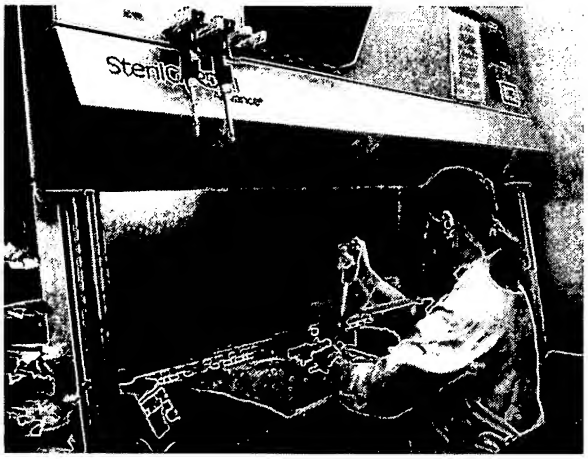
Once the decision is made to introduce a new vaccine or technology, the introduction process must be carefully planned and implementation evaluated in order to avoid the risk of overloading and weakening immunization services. Within five years of introducing a new vaccine, countries will aim to reach coverage levels similar to those achieved with other antigens administered simultaneously in the immunization schedule. Key components in the successful introduction of a new vaccine include: efforts to increase public awareness and education, both on the new vaccine and the disease prevented; health worker training; and the adequate preparation of the logistics and reporting systems. The introduction of new vaccines will require the improvement of surveillance, including the improvement of laboratory facilities. This may require international technical cooperation.

Strategy 9: Activities

- *Integrate the introduction of each new vaccine into countries' multi-year sector-wide plans and provide a financial analysis.*
- *Ensure adequate training of health workers and vaccine managers at all levels and prepare the logistics and reporting systems.*
- *Produce appropriate information, education and communication materials to ensure good understanding of the benefits of new vaccines or technologies, and their acceptance by parents, communities and health workers.*
- *Ensure that within five years of introduction the coverage of the new vaccine reaches the same level of coverage as for other vaccines given at the same time.*
- *Expand surveillance of diseases that can be prevented by new vaccines, and strengthen laboratory capacity to monitor the impact of these new vaccines on disease patterns and programme operations.*

■ Strategy 10: Promote research and development of vaccines against diseases of public health importance

In order to gain the maximum benefits from immunization, efforts are needed to promote the research and development of new vaccines, especially against diseases that cause the highest morbidity and mortality, and to guide the global development agenda for vaccines, technologies and operational research. Countries have a key role to play in defining their priority needs and contributing to the production and analysis of data that will inform and guide the global research agenda. In turn, countries also need to ensure that information generated by research is taken into account in the development of national policies.



It is crucial to continue research and development of new vaccines against diseases that cause the highest morbidity and mortality. *Credit: WHO*

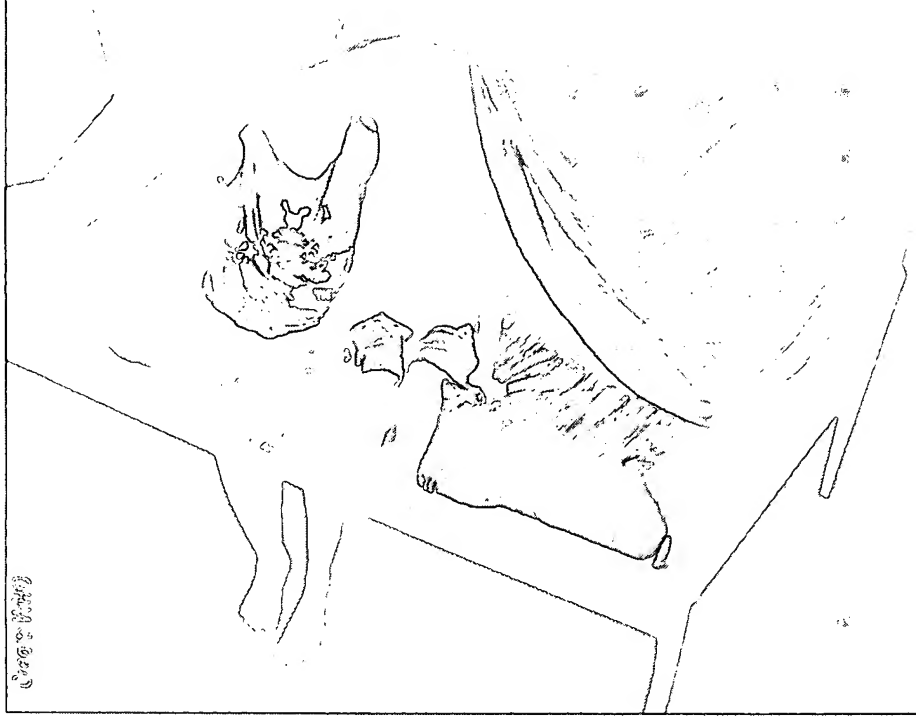
Since the establishment of the WHO/Expanded Programme on Immunization schedule in 1984, many things have changed: polio and measles are on the wane; much more is known about existing vaccines, including the burden of pertussis and, in some countries, diphtheria in older children and adults. Research will be needed to optimize schedules in order to maintain long-term immunity against those infections. New vaccines are being introduced which will target adolescents and young adults, with strategy implications that are not yet clear. As the scope of immunization programmes broadens, there is a need to re-examine the epidemiology of infections for which vaccines exist or will be licensed in the next 5 to 10 years. The characteristics and performance of those vaccines will require carefully designed and conducted clinical trials and post-licensure evaluations, for which capacity in developing countries remains scarce.

Strategy 10: Activities

- *Produce local evidence to influence and prioritize public and private investments in new vaccines and technologies.*
- *Engage local public health authorities and research communities in defining research agendas relevant to countries which bear a disproportionate share of the disease burden.*
- *Strengthen the capacity of developing countries to undertake the research and development of new vaccines and technologies, including conducting high quality clinical trials and post-licensure evaluations.*
- *Generate geographically and epidemiologically representative clinical data on vaccine effectiveness and conduct demonstration projects of post-licensure evaluations of the impact of vaccination on child survival.*
- *Engage the global research and development community, including vaccine manufacturers, in the design and production of new vaccines against infectious diseases of public health importance, especially in developing countries.*
- *Research and develop evidence-based policies for immunization schedules and strategies as new vaccines and vaccine presentations (e.g., vaccine aerosols) and technologies are introduced.*

Strategic Area III

Integrating immunization, other linked health interventions and surveillance in the health systems context



Challenges

Immunization services operate within the health systems context and are, like all health care interventions, profoundly affected by the barriers and challenges encountered by the health system as a whole. The ability of health systems to deliver services such as immunization is often constrained by lack of political and financial commitment, a severe shortage of human resources, inadequate physical infrastructure and equipment, weak monitoring and information systems, lack of management skills, weak social mobilization and previous experience of unmet demand. However, efforts to strengthen immunization services can also help reduce barriers to the equitable delivery of health services and strengthen the health system as a whole. These efforts should be seen as part of a renewed international effort to scale up health services to meet the health-related Millennium Development Goals by 2015.

In the process of health system development, system-wide barriers can be alleviated in areas such as human or financial resources planning and management, outreach services and community mobilization. Immunization can both contribute to these improvements and benefit from them. System-wide barriers such as political and financial commitment (Strategic Area IV), physical infrastructure and equipment (Strategic Areas I and III) and social mobilization (Strategic Areas I and IV) are key areas where immunization both relies on the overall benefits which a sector-wide approach can bring and contributes experience and resources in a range of different areas. The critical area of human resources is another. The lack of well motivated, trained, supervised and adequately paid health staff – compounded in many countries by both AIDS deaths and the impact of the brain drain – is a major constraint on both immunization and the health system as a whole. A sector-wide approach is essential to provide a better career structure and work environment for health workers, as well as improved pay, welfare and other incentives to ensure increased motivation and better deployment of staff. While benefiting from this, immunization services can also contribute through experience in training and effective supervision, as well as in the management of the delivery of services, including the ability to reach populations even in remote, often impoverished, areas. Immunization can also contribute a potential source of trained and experienced health workers from the polio eradication initiative – many of them trained in surveillance activities – who will soon become available for other priority disease activities in developing countries.

Immunization can also help alleviate system-wide barriers by capitalizing on its well-established access to children and women, through linking immunization with the delivery of other essential health interventions. Effective efforts to use immunization contacts as a key opportunity for links with other child survival approaches such as Integrated Management of Childhood Illnesses and the delivery of essential health interventions such as vitamin A, deworming treatments and insecticide-treated nets for malaria prevention would have a rapid impact in reducing child mortality. Immunization is an integral component and often acts as the mainstay of maternal and child health services. Immunization contacts provide a regular opportunity for communities to access additional preventive and curative information, as well as goods and services in support of child health, primary care and reproductive health. For example, vitamin A and other micronutrients, insecticide-treated nets and deworming treatments have all been successfully distributed through both supplementary and routine polio or measles immunization activities.

In addition to the direct health benefits there are other potential advantages in combining targeted health interventions. The expanded provision of locally appropriate, preventive and curative services may result in increased trust by the community in the health system as more of their demands are met. Well planned linkages between interventions may lead to the pooling of human and financial resources and reduce intervention-specific costs if transport and distribution mechan-

isms are shared. The diversification of these services may also have a positive impact on coverage of both immunization and other interventions delivered at the same time. However, careful planning is needed to ensure well-planned and well-resourced linkages in order to avoid excessive demands on peripheral health personnel and unmet expectations. Linkages between health interventions delivered through fixed health facilities and/or outreach services should be systematically planned and documented and, where successful, scaled up. These developments should occur within the context of a wider strategy for strengthening health systems.

Meanwhile, efforts to strengthen surveillance, monitoring and evaluation offer another key opportunity to alleviate system-wide barriers through providing better data to improve health system management. In order to improve the efficiency and effectiveness of surveillance systems at the national level, the management and sharing of data on vaccine-preventable diseases should be part of an integrated surveillance and health information network. The expansion and strengthening of surveillance for vaccine-preventable diseases also provides a critical first line of defence against emerging diseases and diseases of epidemic potential. High performance disease surveillance is needed to detect disease outbreaks, define disease burden, guide policies, strategies and activities, and assess the impact of immunization. Accurate monitoring of immunization coverage is necessary to measure success in delivering vaccines and to determine causes for the continuing incidence of the disease.

Finally, in the event of complex humanitarian emergencies due to conflict or natural disasters – when contact with immunization services is often lost – the early re-establishment of immunization services can contribute to the process of rebuilding damaged health systems. In addition to including immunization in emergency preparedness planning and activities, surveillance for vaccine-preventable diseases should be included in integrated surveillance and monitoring systems established in response to the complex emergency.

New partnerships are being initiated for linking interventions with immunization. *Credit: WHO*

Aims

- Contribute to a sustained enabling environment in the health sector in which every person can be immunized to achieve the disease-control goals set by countries, and where immunization services interact with the entire health sector in a way that enhances the performance of both.
- Address system-wide barriers such as human resource capacity, logistics and overall financial resources, in joint action with all areas of the health sector.
- Explore, plan and implement appropriate linkages between immunization and other interventions tailored to the local context and aim to reach high coverage both for immunization and other child survival activities, and establish joint programme management systems to ensure sustainable linkages.
- Strengthen and expand disease surveillance, coverage monitoring and management information systems to support policy and programme decisions and local action.

The component strategies

■ Strategy 11: Strengthen immunization programmes within the context of health systems development

Opportunities exist to align immunization programmes structurally and functionally within the health system in ways that are mutually strengthening. Focusing on improving capacity at the district level for human or financial resources planning and management, outreach services and community mobilization provides an entry point for the alleviation of system-wide barriers. Efforts will also be needed to identify policy groups and develop collaborative links between them; to establish common understanding around relevant policy contexts; to reach agreement on compatible aims relating to immunization and health-system development; to develop causal links between policy content in immunization and health-system development as a basis for evidence-based policy-making; and to strengthen policy-making structures, systems, skills and values.

Strategy 11: Activities

- *Through regular analysis of district-wide data, document key factors for the success and failure of immunization activities and share these findings with others involved in health systems development.*
- *Participate actively in collective efforts to shape sector-wide policies and programmes, while preserving the central role of immunization in the context of sector-wide policies and programmes.*
- *Use the experience gained in health systems development as an opportunity to position immunization services in a way that ensures the maximum benefit for all people.*

■ Strategy 12: Improve management of human resources

In many countries, the performance of immunization and other health services is hampered by the lack of qualified and experienced staff who are suitably skilled and equipped to reach out to the entire population. The delivery of health services is often undermined by human resource problems such as the inappropriate deployment of staff, inadequate training, low pay and poor supervision. As a result, even with the greatest motivation, health workers may not be able to provide the minimum four contacts per year to the infant population eligible for immunization. Moreover, health staff often have to carry out their duties in conditions of insecurity. Governments have a duty to ensure that appropriate conditions are in place to ensure that health workers are able to perform their work fully, including efforts to minimize the risks to which they are exposed.

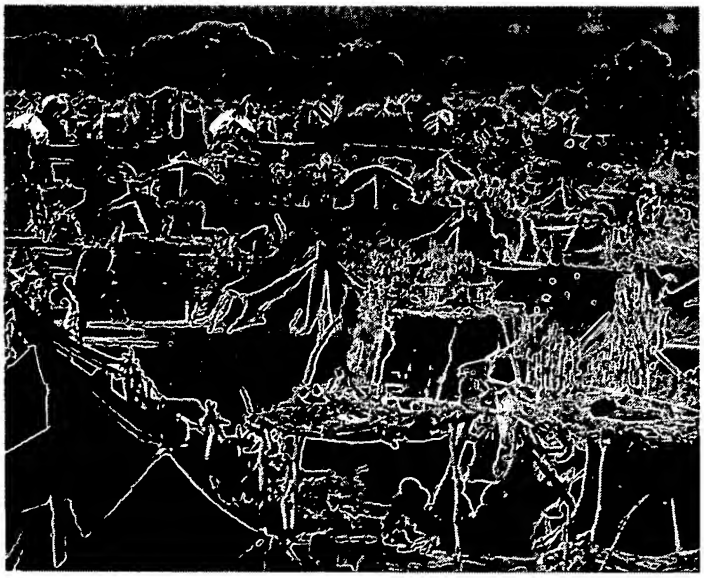
These critical human resource issues need to be addressed not only by immunization programmes but on a system-wide level as well. One potential source of trained and experienced health personnel is the polio eradication initiative which has built up a vast pool of highly committed workers who will gradually become available for other immunization or disease surveillance and control activities as the polio eradication initiative draws to an end. There is a need to tap this wealth of human resources in developing countries. This will require well-performing structures and human resources management so that the contribution of these key health workers can be recognized and they can be redeployed and supported in the long term. This pool of workers should be considered for gradual integration in immunization and the wider health sector to work on other national priority programmes.

Strategy 12: Activities

- *Inventory human resource needs and determine how existing trained immunization personnel can best contribute their skills and experience to new immunization and health systems goals, and engage nongovernmental organizations and the private sector in the delivery of immunization.*
- *Plan for and provide sufficient, adequately paid and trained human resources and match human and financial resources to actual programme needs.*
- *Through improved and secure living and working conditions, training and incentives (including career advancement, improved salaries and family support), motivate health workers in inaccessible or insecure areas to reach all eligible populations.*
- *Ensure that supportive supervision to these health workers is resourced, prioritized, reliably conducted and monitored.*

■ **Strategy 13:** Assess and develop appropriate interventions for integration

Potential linked interventions must be demonstrated to be mutually beneficial, cost-efficient and cost-effective. To meet this need, they should be assessed in terms of their operational and programmatic suitability. In particular, there is a need to consider the opportunity cost and effort both to the person seeking health care and to the health-care provider.



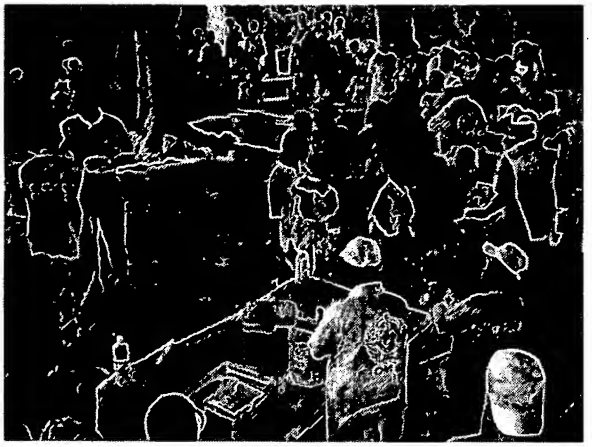
In emergencies, immunization is one of the most cost-effective preventive public health measures.
Credit: WHO

Strategy 13: Activities

- *Develop and field test potential joint interventions according to national and regional priorities to assess their feasibility, safety and potential impact on disease reduction, and document these findings.*
- *Tailor integrated packages of interventions to local needs and feasibility and ensure that they are mutually supportive and designed to meet demand.*
- *At the global level, develop standardized methods for monitoring and evaluating the efficiency, effectiveness and impact of combined interventions, and adapt them for use at the district and service-delivery level.*

■ **Strategy 14:** Maximize the synergy from integrating interventions

Once potential linked interventions have been identified and evaluated, the joint programme activities should be carefully planned and executed to maximize the benefits. Areas of overlap and duplication should be identified and minimized to the benefit of both interventions, and common activities streamlined. Efforts to link immunization with other essential health interventions will lead to improved efficiency in public health services, broaden the partnership base, and contribute to long-term financial sustainability.



Women and their children queue up to register at an immunization post. The children receive four life-saving interventions at once – polio and measles vaccination, deworming tablets and insecticide-treated nets to prevent malaria. *Credit: UNICEF*

Strategy 14: Activities

- *Include joint interventions in multi-year and annual plans, ensuring the acceptance and participation of all stakeholders within the programmes, the district management teams and the community.*
- *Formulate and implement as part of these plans, integrated training plans based on training needs assessments and appropriately developed training material.*
- *Implement interventions jointly, choosing from fixed, outreach, mobile, Child Health Day and supplementary immunization activity approaches. Special emphasis should be placed on outreach and mobile teams in situations where they represent the best means of contact between hard-to-reach populations and health services.*
- *Monitor and evaluate the incremental efficiency, effectiveness and impact of combined interventions and their means of delivery; apply these findings in order to continuously improve the combined intervention, increase the range of joint interventions, and contribute to long-term financial sustainability.*

■ **Strategy 15:** Sustain the benefits of integrated interventions

In the long term, links between priority child survival interventions and approaches, including Integrated Management of Childhood Illnesses, and immunization services will require the ultimate harmonization of policy and programme development and implementation, as well as resource allocation.

Strategy 15: Activities

- *Bridge different programmes in global agencies and within countries by formalizing a management structure that facilitates coordination and efficiency without disregarding programme-specific needs.*
- *Establish joint financing, monitoring and evaluation functions.*
- *Pool the resources needed to cover operational and other costs.*
- *Remain attentive to community-perceived needs and provide quality information to secure sustained community support.*
- *Advocate for further synergy and explore additional linkages.*

Strategy 16: Strengthen monitoring of coverage and case-based surveillance

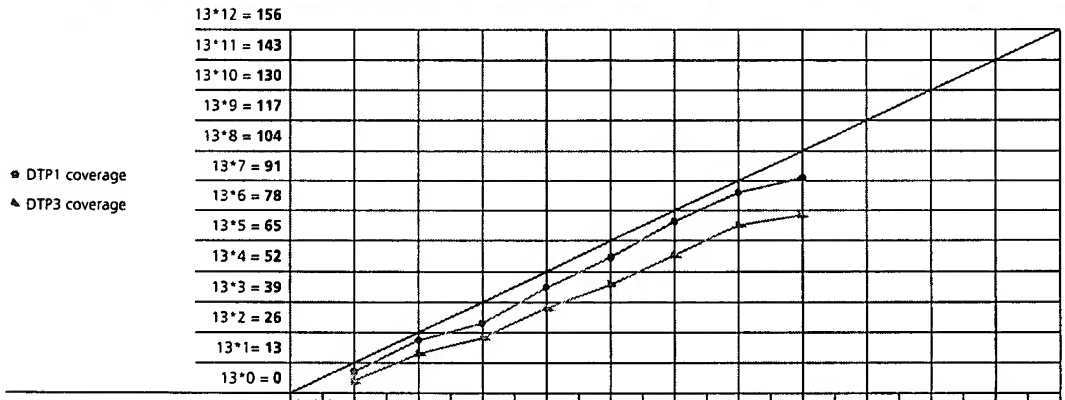
Coverage monitoring and case-based surveillance are central to programme management. Lack of data as well as poor quality data and data analysis are well known system-wide barriers. Both surveillance and coverage monitoring require efforts to build human capacity for field surveillance and for the collection, compilation, analysis, interpretation and use of data. Vaccine coverage and other monitoring systems can be improved through data quality self-assessments, better systems to compile and analyse data, and regular feedback to district and local levels. In addition, the expansion and strengthening of vaccine-preventable disease surveillance can ultimately be used to detect emerging infections and other priority diseases, and contribute to strengthening overall disease surveillance.

The introduction of new vaccines and technologies will require the expansion of existing surveillance systems to include diseases prevented by new vaccines, and the strengthening of laboratory capacity to monitor the impact of these new vaccines. As resources and laboratory-testing capacity increase, surveillance for new

Strategy 16: Activities

- *Expand the existing surveillance systems (such as polio and measles surveillance) in order to progress towards effective case-based surveillance for vaccine-preventable diseases, i.e., both existing vaccine-preventable diseases and diseases for which vaccines are anticipated.*
- *Improve coverage monitoring of vaccines and other linked health interventions and the use of information at district and local levels through strengthening human resource capacity, monitoring the quality of data, improved tools for data compilation, feedback and supervision.*
- *At the global level, develop and provide countries with new methodologies to estimate the burden of disease in order to obtain more accurate estimates of disease and to monitor vaccination coverage and programme performance towards achieving national, regional and global goals.*

Figure 4: Example of an immunization monitoring chart



Fill in at the end of each month	JAN	Cum. Tot.	FEB	Cum. Tot.	MAR	Cum. Tot.	APR	Cum. Tot.	MAY	Cum. Tot.	JUNE	Cum. Tot.	JULY	Cum. Tot.	AUG	Cum. Tot.	SEPT	Cum. Tot.	OCT	Cum. Tot.	NOV	Cum. Tot.	DEC	Cum. Tot.
Total immunized DTP1	10	10	12	22	7	29	12	41	14	55	15	70	14	84	7	91								
Total immunized DTP3	8	8	9	17	8	25	10	35	11	46	12	58	12	70	7	77								
Drop out number (DO) (DTP1-DTP3)		2		5		4		6		9		12		14		14								
Drop out % (DO/DTP1)*100		20		23		14		15		16		17		17		15								

Cum. Tot. = Cumulative total

Source: WHO

vaccines will eventually become case-based, with laboratory confirmation where appropriate. Surveillance needs to be implemented well in advance of the introduction of any new vaccine in order to provide information on disease burden, serotype prevalence and vaccine selection, as well as the baseline information needed to judge the effectiveness of the vaccine.

Surveillance, monitoring and evaluation are integral components of successful immunization systems, but require a system-wide infrastructure to succeed. This includes adequate transport, communications, materials, methods and logistics for specimen collection and dispatch, as well as funds for operating expenses for surveillance.

■ **Strategy 17:** Strengthen laboratory capacity through the creation of laboratory networks

Laboratory capacity and networks will be strengthened in order to ensure their ability not only to confirm cases of vaccine-preventable diseases but also to determine the causes and establish the disease burden of other priority diseases. National laboratories will be an integral component of regional networks. A basic infrastructure and logistics system will be vital and can be expanded for other vaccine-preventable diseases by expanding existing laboratory networks and by ensuring adequate transport, communications, materials, methods and logistics for specimen collection and dispatch, and funds for operating expenses and quality control procedures.

Strategy 17: Activities

- *Expand the existing laboratory networks (including the polio and measles laboratory network and other regional and local networks such as the Paediatric Bacterial Meningitis Network and the networks established by GAVI's Accelerated Development and Introduction Plans for pneumococcal and rotavirus vaccines) to include other priority diseases.*
- *Assure the training, equipment, reagents and quality control procedures needed to sustain high quality diagnostics for all vaccine-preventable diseases and other priority diseases.*
- *At the global level, develop new diagnostic tests, tools and procedures to improve both field-based and laboratory confirmation of diagnoses.*

■ Strategy 18: Strengthen the management, analysis, interpretation, use and exchange of data at all levels

The management and sharing of information underpin all surveillance, monitoring, and evaluation components. At the country level, data management for vaccine-preventable diseases should be part of an integrated surveillance and health information framework with the potential to provide information on a range of national priority diseases. Data management will be strengthened through capacity building, training and the use of frequent and regular supervision. Appropriate tools will be developed for local use, which will facilitate standardized and ad hoc data analyses. National immunization programmes should seek linkages with other health programmes (e.g., Integrated Management of Childhood Illnesses, malaria, HIV/AIDS and tuberculosis) to improve the national health information systems.

Efforts are under way to improve the efficiency and timeliness of the registration of births and deaths (vital registration) in an effort to ensure that more children are able to benefit from health care, education and other public services to which they are entitled. Immunization services can use these data to monitor coverage and impact as they do in countries where birth registration achieves high coverage. In turn, the increasing demand for immunization may motivate parents to register their children soon after birth.

Strategy 18: Activities

- *Contribute to the design of integrated management information systems and improve data management through regular training, monitoring and feedback at the local level.*
- *Regularly review district indicators of performance, including risk status for vaccine-preventable diseases and use surveillance and monitoring data to advocate for improved access to, and quality of, immunization.*
- *Contribute to the development of better tools (e.g., computer software) for monitoring coverage of vaccines and linked interventions, vaccine and logistics management, and disease surveillance to better support data entry, analysis, feedback, and utilization for programme management.*
- *Monitor the quality and performance of coverage monitoring and surveillance systems through surveys, monitoring of performance indicators, data quality assessments, disease modelling and supportive supervision.*
- *Collaborate with civil authorities in advocating for increased registration of births and deaths.*

■ **Strategy 19:** Provide access to immunization services in complex humanitarian emergencies

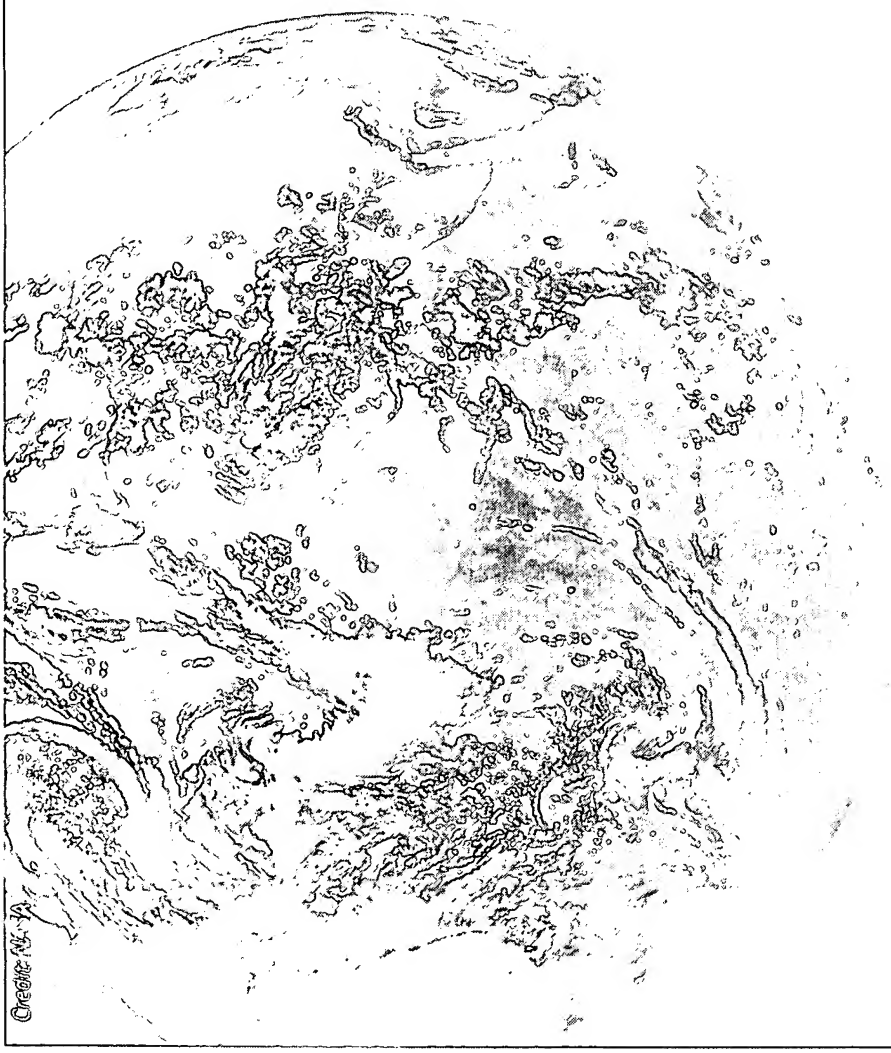
In complex emergencies such as wars, natural disasters and civil conflict, immunization can play a critical role in preventing disease and deaths in already difficult circumstances, as well as preventing the potential spread of disease to neighbouring populations. This timely intervention can also provide an early signal of humanitarian concern for populations under stress and lays the cornerstone for the rehabilitation or reconstruction of devastated health systems.

Since emergencies and disasters are, by their very nature, difficult to predict, adequate contingency plans and appropriate supplies should be maintained at global and regional levels.

Strategy 19: Activities

- *Include immunization-related issues in rapid situation assessment of complex emergencies.*
- *Incorporate immunization services in emergency preparedness plans and activities.*
- *Re-establish immunization services in populations affected by complex emergencies and link these services to the rehabilitation of health systems.*
- *Create global capacity to advise on appropriate immunization strategies in complex emergencies and natural disasters.*
- *Include vaccine-preventable diseases in integrated surveillance and monitoring systems established in response to complex emergencies.*

Strategic Area IV
Immunizing in the context of global interdependence



Challenges

The interdependence between low-income, middle-income and high-income countries, between sectors of human development, and across components of immunization programmes, requires a global vision of how resources such as the global supply of vaccines can best be secured and used equitably. Components that have proven to be essential to ensure an adequate global supply of assured quality vaccines include accurate forecasts, financial planning, appropriate contracting, and adequate stock management and distribution systems.

A continuing issue of concern is the divergence of vaccine markets which has occurred mainly due to factors such as cost, differences in disease burden and concerns about possible side-effects. As a result, developing countries today increasingly provide vaccine antigens or vaccine formulations that are different to those provided in industrialized countries. This divergence has profound implications for the grouped procurement systems operated by UNICEF and the Pan American Health Organization (PAHO). These systems enable national immunization programmes in developing countries to obtain vaccines at reduced prices and to be included in aggregated short-term and long-term forecasts to industry thereby helping to ensure the availability of vaccines. However, these systems will face difficulty in procuring increasingly diversified and costly products, well in advance of their use, when their introduction remains heavily dependent on both reliable forecasting of national needs and efficiency in implementation.

As many new and more costly vaccines become available in the future, governments will face hard choices about which vaccines to include in the national immunization schedule. Efforts to determine whether a vaccine is "affordable" or not will depend very much on the perceived value of vaccines which, even at higher prices, are expected to remain a highly cost-effective intervention. A key challenge over the next 10 years will be to reduce the financial barriers to high population coverage with existing and new vaccines, and to ensure that both governments and partners adopt policies and take actions that favour stable and adequate financing for vaccines and immunization in the future.

Appropriate co-financing strategies will be developed to reduce the financial barriers to the introduction of new and existing vaccines and technologies. In addition, efforts will be made to ensure that external financial support to countries is provided in ways that strengthen national capacity to gradually assume financial responsibility for immunization. GAVI/VF have a key role to play in this effort.

The dramatic changes in the information and communications environment that have occurred globally over the past decade – mainly due to the globalization of the media and pervasive access to Internet-based information – have had both a positive and a negative impact on public perceptions about vaccines. The frequency of concerns about vaccine safety has increased as interested parties share their concerns globally and, in turn, appreciation of the value of immunization appears to have waned. On the other hand, information on the benefits of immunization can now reach more people more rapidly and help ensure that caregivers are better informed about the choices and options available to them.

Finally, global interdependence has increased the vulnerability of people everywhere to the uncontrolled spread of diseases through epidemics of national or global proportions. Infectious diseases can easily and quickly spread from country to country (e.g. polio). Vaccines already exist to prevent or control some of these diseases, and others are likely to become available in the next decade. Local, national and global preparedness against epidemics has become a critical step in safeguarding global health and security.

An illustrative example

How much will it cost?

Costing an immunization vision is not a straightforward exercise. Any estimate will be subject to uncertainty in the data and methods used to estimate costs, as well as in the selection of interventions each country would choose to achieve immunization goals set forth in the global strategy. These costs will also be sensitive to the availability and future prices of underused and new vaccines, as well as the availability of funds to finance continuous expansions and improvements of immunization systems.

Given these uncertainties, and based on existing data and methods, preliminary estimates were made of current spending on immunization, as well as the cost of scaling up immunization efforts between the years 2006 and 2015 in 117 of the poorest countries.[a] The impact that scaling up immunization could have on the mortality attributable to vaccine-preventable diseases was also estimated.

The broad assumptions of this illustrative example are that each country will aim to reach 90% routine coverage by 2015, including campaigns as appropriate;[b] and will introduce available and safe vaccines, including new vaccines expected to be widely available during the period 2006–2015.[c]

Results suggest that in the year 2000, approximately US$ 1 billion was spent on routine immunization for the delivery of basic vaccines. By 2006, costs are expected to have doubled as countries expand their immunization schedules with underused vaccines and accelerated measles mortality reduction campaigns. By 2015, the annual costs of reaching all the goals outlined in the illustrative example are estimated to be three times those of 2006.

Achieving the goals outlined in the illustrative example will require at least a doubling of current financing for immunization if a continuation of current investments by national governments and external donors, as well as new international funding for immunization (e.g., GAVI), are assumed.

However, such costs are not without a good return on investment, particularly in terms of the contribution to achieving the Millennium Development Goal on child mortality reduction (MDG 4). In 2000, immunization prevented 1–2 million child deaths in a single year. By 2015, this will more than double, preventing 4–5 million child deaths per year. The full benefits of sustaining current immunization efforts – and scaling them up – could prevent more than 38 million premature deaths in 2006–2015. At an average cost of under US$ 1000 per death averted, immunization continues to be one of the best health investments available.

The numbers cited above should be considered as indicative and preliminary in nature. Efforts are ongoing to improve these estimates.[d] More definitive figures will be available in 2006.

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a. All costs referred to include shared health systems costs.

b. Includes campaigns for rapid mortality reduction (tetanus, measles) in conjunction with the introduction of new or underused vaccines (rubella, yellow fever, and meningococcal A). Also included are the costs of the final stages of polio eradication, the bulk of which is for campaigns.

c. Includes Hib, hepatitis B, yellow fever, rubella and second dose measles vaccines as "underused vaccines", and rotavirus, pneumococcal, meningococcal A and Japanese encephalitis vaccines as "new vaccines". The vaccine costs for provision of a booster dose of DTP are not included. It is assumed that oral polio vaccine use will cease in 2010 and any costs associated with the use of inactivated polio vaccine are not included.

d. Further methodological details are available in: Wolfson L, and Lydon P, *Methods for estimating global immunization costs and Impact, 2005–2015*, Geneva, World Health Organization, 2006.

Aims

- Increase awareness of and respond to the reality that every country is vulnerable to the impact of global issues and events on vaccine supply, financing, partner collaboration, communication and epidemic preparedness.
- Strengthen and coordinate partnerships at global and national levels in support of immunization programmes.

The component strategies

■ Strategy 20: Ensure reliable global supply of affordable vaccines of assured quality

The success of immunization depends on a sustainable supply of affordable vaccines of assured quality. Inadequate vaccine procurement, inaccurate forecasting and late ordering create obstacles for the immunization programme. Collaboration between countries, vaccine manufacturers and international supply agencies should be strengthened to ensure that global demand is identified and actions taken to meet that demand.

Ultimately, the price of vaccines will, to some extent, depend on the ability of the global community to address the difficult process of forecasting vaccine needs and making available the appropriate vaccine antigens and formulations – a strategy which requires effective partner and country interaction.

Strategy 20: Activities

- *Ensure long-term forecasting for existing and new vaccines through close collaboration between international agencies, donors and vaccine manufacturers.*
- *Develop global standards and methods for testing the quality, safety and efficacy of vaccines and other biologicals.*
- *Promote the production of affordable vaccines of assured quality by vaccine manufacturers in developing and developed countries.*
- *Promote the emergence of multiple manufacturers from industrialized and developing countries to provide an adequate supply of affordable vaccines of assured quality (both existing and new vaccines) and immunization materials.*

Vaccines, autodisable syringes and safety boxes are now regularly "bundled" together from supplier to point of use. *Credit: WHO*

■ Strategy 21: Ensure adequate and sustainable financing of national immunization systems

Achieving financial sustainability for immunization, in order to sustain gains in disease reduction, improve coverage and introduce new products, will require the mobilization of new resources both from within countries and from the international community. The principles or criteria for donor participation in the funding process need to be delineated. In addition, clear criteria and principles are needed to define the appropriate interactions between partners and national immunization programmes when the introduction of new or underutilized vaccines and/or technologies is under consideration.

Strategy 21: Activities

- *Strengthen national capacity for financial planning both within the immunization programme itself and the ministry of health as a whole.*
- *Commit increased and sustained national budget allocations for vaccines on the basis of improved understanding of the value of vaccines in public health.*
- *Encourage local and district level contributions to health services and immunization programmes through interaction with local businesses and interests.*
- *Mobilize international solidarity to secure and sustain financing for immunization, including long-term commitments by existing public and private funding entities and new long-term financial mechanisms in support of the research, development, production and use of new vaccines.*
- *Coordinate immunization financing through the Interagency Coordinating Committees to ensure adequate and appropriate donor support to national governments.*

■ Strategy 22: Improve communication and dissemination of information

Communication must be improved in order to ensure that the public, policy-makers, and health workers understand the vital importance of immunization for the health of both children and adults. This is essential both in ensuring support for the current immunization programme and in providing information about the introduction of new vaccines or technologies to a national schedule. As delivery systems become more complex and the diversity of available products increases, the demand for clear guidance on programme preferences will also intensify. In view of the globalization of the media, including widespread access to Internet-based information, it is of critical importance to make use of the available media both to provide evidence-based information about the value of immunization and to counter false information about vaccine safety issues.

Strategy 22: Activities

- *Develop new ways of using the globalized media, including the Internet, to build public awareness of the benefits of immunization.*
- *Produce quality and timely information on the benefits of immunization and associated risks, and develop key messages to promote immunization according to national needs and priorities.*
- *Through regional and global channels, document and systematically communicate the experience gained by countries that have added new vaccines and technologies.*

■ **Strategy 23:** Define and recognize the roles, responsibilities and accountability of partners

Global partners should provide leadership and coordination, particularly in setting global goals, lead global advocacy efforts to ensure that immunization remains high on the international health agenda, and support research to facilitate the introduction of new vaccines and technologies and to improve immunization programmes. Immunization partners also play an important role at national and regional levels through various partner coordinating mechanisms such as the Interagency Coordinating Committees.

Strategy 23: Activities

- *Negotiate and define the roles and responsibilities of key immunization partners at the global level on a regular basis to ensure both accountability and efficient coordination.*
- *Set global immunization goals jointly and in consultation with countries to ensure the full commitment of all parties.*
- *Obtain global concurrence on policies, norms and standards for immunization and additional interventions.*
- *Provide leadership in global advocacy and ensure that immunization remains high on the global health agenda by raising awareness of the importance and benefits of immunization among governments and donors and in the global community.*
- *Develop and actively participate in regional and national partnership bodies (such as Interagency Coordinating Committees) to support implementation, provide ongoing technical assistance and monitor progress in countries.*
- *Support epidemiological and operational research on vaccines and immunization.*

■ **Strategy 24:** Include vaccines in global epidemic preparedness plans and measures

Vaccine-preventable diseases may occur in epidemic form, whether localized or affecting a region, a country or the whole world. Of the vaccines available to prevent or contain such epidemics, some are insufficiently used (e.g., yellow fever in endemic countries), others are under improvement (e.g., Japanese encephalitis, cholera), and others may be made available only in limited quantities and after the time required for their formulation and production (e.g., influenza). In addition, other vaccines against diseases of epidemic potential may become available by 2015 (e.g., severe acute respiratory syndrome [SARS] and dengue). In line with their commitment to strengthen epidemic alert and response capacity, countries and international organizations are in the process of formulating national preparedness plans for epidemic control. However, developing countries have a number of structural and functional obstacles to overcome before they become fully prepared to face such events. Immunization programmes are important potential contributors to epidemic preparedness and response at the national and global levels through: surveillance; vaccine procurement and delivery; promotion of safe immunization practices and logistic outreach.

Strategy 24: Activities

- *Develop global and country-specific epidemic preparedness and prevention plans relevant to specific diseases.*
- *Develop and implement plans and funding for a stockpile of key vaccines for both epidemic control and pre-emptive campaigns.*
- *Maintain an effective surveillance system linked to the Global Alert and Response Network enabling the appropriate and timely use of vaccines in the context of emerging or threatening epidemics, and share information globally.*
- *Strengthen regulatory capacity to respond to urgent needs for epidemic preparedness and response.*

The way forward

The way forward

This section focuses on the actions needed to facilitate the global implementation of the global strategy: consultations to ensure that countries apply the guiding principles to their own strategic planning through strategies tailored to individual needs, capacity and resources; securing the early engagement of immunization partners; concerted strengthening of the capacity of immunization services at the district level, especially in low-performing countries; establishment of a knowledge base about successfully linked health interventions as a resource for their potential scaling up; development of an evaluation and review process to measure progress up to 2015; and production and dissemination of supportive documentation detailing plans and policies, as well as further information on technical issues.

Consensus development and national commitment

National and regional consultations are already using the global strategy as a basis for elaborating policies and plans tailored to their specific needs, capacity and resources. In doing so, countries will require the commitment of governments, including not only ministries of health but also ministries that oversee planning, finance, education and local government, and have a key role to play in health matters. It will require the engagement of programme managers as well as the close involvement of partners from civil society and the private commercial sector. Underlying assumptions for progress towards the global strategy goals include efforts involving partnerships at all levels to improve management, develop and implement appropriate advocacy strategies, and monitor and evaluate results. While these partnerships have a significant role to play at the regional and global levels, it is at the country level that they can have a major impact. In an effort to maximize the value of this role, crucial efforts will be needed to strengthen the Interagency Coordinating Committees in order to improve coordination.

Strengthening district capacity for implementation of the global strategy with a focus on low-performing countries

The implementation of the global strategy relies on efforts at the district level. To meet this need, support will be needed at the district level to help identify and overcome constraints on the delivery of immunization, to deploy human and financial resources in ways that best meet local needs, and to use local data to inform such decisions. A key priority in district plans and activities will be efforts to reach out to underserved communities and to engage the participation of communities in these efforts.

Countries have reached different stages of progress towards their immunization goals. Those that have demonstrated their capacity to improve and sustain the coverage and quality of immunization will deserve continued support for their activities as they further improve their performance and introduce new vaccines. Special efforts will be devoted to low-performing countries in order to accelerate progress. To this end, national, regional and global consultations will set priorities for action, taking into account the extent of national commitment and gaps in immunization delivery, implementation capacity and financing. In low-performing countries, concerted action by national and international stakeholders will seek to alleviate constraints affecting not only the performance of immunization services but the health system as a whole.

Experimentation and scaling up of integrated interventions

In areas of proven integration (e.g., malaria control and routine immunization), countries and partners are encouraged to actively pursue and support joint planning, implementation and monitoring of these activities.

Building on new knowledge derived from operational research and practical experience, new operational linkages will be created between immunization and other health interventions. Combined interventions which have proven to be efficient, effective and sustainable will be replicated on a wider scale and new pathways explored to further enhance the catalytic role of immunization. Joint management, financing, monitoring and evaluation mechanisms for linked interventions will be explored to ensure improved coordination, both within immunization programmes and between them and other health-sector initiatives.

Evaluation, review and adjustment

Over the 10-year period covered by the global strategy, periodic progress reviews and selected probes into specific issues arising in the course of implementation will inform the shaping and re-shaping of policies and programmes. A mid-term evaluation of the global strategy will be conducted in 2010 and an end-term review in 2015.

The reliable evaluation of programme performance and impact will require the improvement of current monitoring and evaluation methods as

A health worker carrying a vaccine cold box on the back of a bicycle. Bicycles are a common mode of transport and a vital way to distribute vaccines. *Credit: UNICEF*

well as the development of new ones. Moreover, efforts will be needed to strengthen national and international capacity to apply appropriate and up-to-date monitoring and evaluation methods. Such efforts will result both in the establishment of new measurable targets, against which progress can be reliably measured, and in greater accountability for the use of national and international resources.

Development of accompanying documents

Several frameworks and instruments will be needed to translate the vision and strategies laid out in this document into national or institutional policy, planning, implementation, monitoring and evaluation. These will include the following:

- companion technical documents for national use describing in greater detail key issues such as comprehensive multi-year planning, including financing; human resources planning; medium-term and long-term vaccine procurement forecasts; priorities for research and development; progress and impact indicators; and monitoring and evaluation frameworks;
- projected global financial needs and consolidated monitoring and evaluation targets and indicators, based on the range of national plans and budgets;
- formulation of national and regional comprehensive multi-year strategic plans that set targets, define strategies and provide human and financial plans, emphasizing accountability and the decentralization of decision-making authority and resources to the district level;
- a global plan for reliably monitoring the goals of the global strategy through an approach that builds sustainable monitoring capacity within the country, particularly at the district level; such a plan would outline a strategic approach for strengthening coverage monitoring, surveillance, and laboratory capacity for vaccine-preventable diseases by building on existing systems within countries and, at the same time, emphasizing high performance and accuracy;
- national, regional and global financing plans; and
- a global development and research strategy responding to the current and anticipated needs of the global strategy.

The strategic options outlined above are not exhaustive. The global strategy should be seen not as a detailed blueprint but rather as an evolving plan. As the strategy and vision unfold over the next 10 years, new challenges will arise and new responses and innovations will be needed.

Annexes

Annex 1

World Health Organization

World Health Assembly resolution, 2005

FIFTY-EIGHTH WORLD HEALTH ASSEMBLY — WHA58.15

Agenda item 13.8 — 25 May 2005

Draft global immunization strategy

The Fifty-eighth World Health Assembly,

Having considered the report on the draft immunization strategy;*

Alarmed that globally and in some regions immunization coverage has increased only marginally since the early 1990s, and that in 2003 more than 27 million children worldwide were not immunized during their first year of life;

Recognizing that each year 1.4 million children under five years of age die from diseases preventable by currently available vaccines;

Further recognizing that each year an additional 2.6 million children under five years of age die because of diseases potentially preventable by new vaccines;

Acknowledging the contributions by WHO, UNICEF, GAVI and all partners in their efforts in strengthening immunization services, expansion of immunization coverage and introduction of new and underused vaccines in developing countries;

Welcoming the achievements of the accelerated disease-control initiatives against poliomyelitis, measles, and maternal and neonatal tetanus in immunizing previously unreached populations, and noting that these initiatives have established extensive networks on which surveillance for other disease and health trends can be built or expanded;

Concerned that, owing to financial, structural and/or managerial constraints, national immunization programmes fail to reach all who are eligible for immunization, particularly children and women, underuse many existing vaccines, and are not widely introducing new vaccines;

Emphasizing the need for all countries to strive towards achieving the internationally agreed development goal in the United Nations Millennium Declaration of reducing by two thirds, between 1990 and 2015, the under-five child mortality rate;

Recalling the target of the United Nations General Assembly's twenty-seventh special session on children (2002) to ensure full immunization of children under one year of age, with at least 90% coverage nationally, and at least 80% coverage in every district or equivalent administrative unit;

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* Document A58/12.

Recognizing that resolution WHA53.12 highlights immunization as a major factor in promoting child health;

Having considered the draft global immunization vision and strategy,

1. WELCOMES the Global Immunization Vision and Strategy;
2. URGES Member States:
   (1) to meet immunization targets expressed in the United Nations General Assembly special session on children;
   (2) to adopt the Global Immunization Vision and Strategy as the framework for strengthening of national immunization programmes between 2006 and 2015, with the goal of achieving greater coverage and equity in access to immunizations, of improving access to existing and future vaccines, and of extending the benefits of vaccination linked with other health interventions to age groups beyond infancy;
   (3) to ensure that immunization remains a priority on the national health agenda, and is supported by systematic planning, implementation, monitoring and evaluation processes, and long-term financial commitment;
3. REQUESTS the Director-General:
   (1) to mobilize resources to promote the availability and affordability in countries of future new vaccines based on evidence of epidemiological profiles;
   (2) to work closely with the Global Alliance for Vaccines and Immunization (GAVI), UNICEF and other partners to provide support to Member States in implementation of the Global Immunization Vision and Strategy;
   (3) to strengthen relations at global, regional and subregional levels with UNICEF, GAVI and other partners in order to mobilize the needed resources for countries, in particular developing countries, to implement the Global Immunization Vision and Strategy;
   (4) to report every three years to the Health Assembly on progress towards achievement of global immunization targets, including those expressed in the United Nations General Assembly special session on children.

Ninth plenary meeting, 25 May 2005
A58/VR/9

Annex 2

unicef

UNICEF Executive Board decision, 2005

UNICEF/WHO Global Immunization Vision and Strategy

Executive Board Decisions – Programme (2005 Annual Session)

Document Symbol/Series:	2005/7
Country:	Global
Date:	9 June 2005
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Executive Summary:

Document Text:

2005/7. UNICEF/WHO Global Immunization Vision and Strategy

The Executive Board,

Having considered the report on the draft immunization strategy (E/ICEF/2005/9 and WHO A58/12),

Alarmed that globally and in some regions immunization coverage has increased only marginally since the early 1990s, and that in 2003 more than 27 million children worldwide were not immunized during their first year of life;

Recognizing that each year 1.4 million children under five years of age die from diseases preventable by currently available vaccines;

Further recognizing that each year an additional 2.6 million children under five years of age die because of diseases potentially preventable by new vaccines;

Welcoming the achievements of the accelerated disease-control initiatives against poliomyelitis, measles, and maternal and neonatal tetanus in immunizing previously unreached populations, and noting that these initiatives have established extensive networks on which surveillance for other disease and health trends can be built or expanded;

Concerned that, owing to financial, structural and/or managerial constraints, national immunization programmes fail to reach all children and women eligible for immunization, underuse many existing vaccines, and are not widely introducing new vaccines;

Emphasizing the need for all countries to strive towards achieving the internationally agreed development goal in the United Nations Millennium Declaration of reducing by two thirds, between 1990 and 2015, the under-five child mortality rate;

Recalling the target of the United Nations General Assembly's twenty-seventh special session on children (2002) to ensure full immunization of children under one year of age, with at least 90% coverage nationally, and at least 80% coverage in every district or equivalent administrative unit;

Having considered the draft global immunization vision and strategy,

1. *Welcomes* the Global Immunization Vision and Strategy;
2. *Urges* countries:
   (a) to meet immunization targets expressed in the United Nations General Assembly Special Session on Children;
   (b) to adopt the Global Immunization Vision and Strategy as the framework for strengthening of national immunization programmes between 2006 and 2015, with the goal of achieving greater equity in access to immunization, of improving access to existing and future vaccines, and of extending the benefits of vaccination linked with other health interventions to age groups beyond infancy;
   (c) to ensure that immunization remains a priority on the national health agenda, and is supported by systematic planning, implementation, monitoring and evaluation processes, and long-term financial commitment;
3. *Requests* the Executive Director:
   (a) to work closely with the World Health Organization (WHO), Global Alliance for Vaccines and Immunization (GAVI), and other partners to provide support to Member States in implementation of the Global Immunization Vision and Strategy;
   (b) to strengthen relations at global, regional and subregional levels with WHO, GAVI and other partners in order to mobilize the needed resources for countries to implement the Global Immunization Vision and Strategy;
   (c) to report regularly to the Executive Board on progress towards achievement of global immunization targets.

Annual session

9 June 2005

Annex 3

GIVS framework

Protecting more people in a changing world

GIVS strategies	*Activities*
Strategy 1: Use a combination of approaches to reach everybody targeted for immunization	Strengthen national commitment to ongoing immunization services through policy and strategy development that also includes human resources and financial planning with national budget allocations, in the context of a wider health sector strategic plan.
	Formulate and implement comprehensive multi-year national strategic plans and annual workplans to deliver reliable services based on data analysis and problem solving. The plans and budgets should cover all areas supporting the national immunization programme, including routine vaccination, accelerated disease-control activities, introduction of new vaccines, surveillance, laboratory support, financing logistics, vaccine management, cold-chain management, social mobilization and communication.
	Sustain high vaccination coverage where it has been achieved, by ensuring that adequate support is maintained for existing systems.
	Develop appropriate national strategies to immunize children who were not immunized during infancy.
	Where and when appropriate, include supplementary immunization activities as an integral part of the national plans to achieve national goals.
Strategy 2: Increase community demand for immunization	Engage community members, nongovernmental organizations and interest groups in immunization advocacy and implementation.
	Assess the existing communication gaps in reaching all communities and develop and implement a communication and social mobilization plan as part of the comprehensive multi-year plan based on these assessments. The plan should include ways of targeting unreached communities, establishing well-informed community demand, and addressing the problem of immunization refusal.
	Provide regular, reliable, and safe immunization services that match demand.

GIVS strategies	*Activities*
Strategy 3: Ensure that unreached people are reached in every district at least four times a year	☐ Through microplanning at the district or local level, map (geographically, socially, culturally) the entire population in order to identify and reach the unreached target populations at least four times a year.
	☐ Reduce the number of immunization drop-outs (incomplete vaccination) through improved management, defaulter tracing, and social mobilization and communication during immunization contacts, and avoid missed opportunities to vaccinate.
	☐ Strengthen the managerial skills of national and district immunization providers and managers and develop and update supervisory mechanisms and tools.
	☐ Provide timely funding, logistic support and supplies for programme implementation in every district.
Strategy 4: Expand vaccination beyond the traditional target group	☐ As part of national policy and strategy development, define target populations and age groups for vaccination appropriate to the national situation, making the protection of those outside the infant age group an integral component of immunization services.
	☐ Apply standard tools to assess the cost-effectiveness of different immunization schedules and strategies in a range of demographic, geographic and epidemiological settings.
Strategy 5: Improve vaccine, immunization and injection safety	☐ Procure vaccines only from sources that meet internationally recognized quality standards.
	☐ Ensure long-term forecasting for existing and new vaccines by improving vaccine-management skills.
	☐ Achieve national self-reliance in quality assurance and regulatory oversight to meet global standards, and promote and further strengthen existing programmes that support this (in particular, scientific evaluation, capacity building, public education, training and communication).
	☐ Introduce, sustain and monitor safe injection practices, including the use of autodisable syringes and other safe methods of vaccine administration, and thereby contribute to the enforcement of safe injection practices and health-care waste disposal.
	☐ Establish surveillance and response to adverse events following immunization, both for existing vaccines and for new vaccines as they are introduced into national schedules.
	☐ Be responsive to potential vaccine safety issues and address these urgently.

GIVS strategies	*Activities*
Strategy 6: Improve and strengthen vaccine-management systems	▪ Conduct accurate demand forecasting at national and district levels to ensure the uninterrupted supply of assured quality vaccines, autodisable syringes and safety boxes, and new types of equipment as they become available. Forecasting should be reviewed regularly to respond to changing delivery strategies.
	▪ Build capacity for effective vaccine management through training, supervision and the development of information systems in order to ensure the safety and potency of vaccines up to the point of use.
	▪ Increase access and coverage through a "safe chain" approach which includes taking vaccines beyond the cold chain, using a vaccine vial monitor-based system for vaccine-management.
	▪ Move towards coordinated and sector-wide financing and management for transportation and communications.
Strategy 7: Evaluate and strengthen national immunization programmes	▪ Conduct regular immunization programme evaluations at local, district and national levels and provide feedback on performance, obstacles and new opportunities to all partners.
	▪ Where appropriate, perform operations research and evaluation of "what works" to improve the delivery of immunization and to make systems more effective, efficient and equitable in order to improve immunization coverage.

Strategic Area II: Introducing new vaccines and technologies

GIVS strategies	*Activities*
Strategy 8: Strengthen country capacity to determine and set policies and priorities for new vaccines and technologies	☐ Strengthen country capacity to assess disease burden and the cost and cost-effectiveness of new vaccines and technologies through the use of standard tools. ☐ Characterize the optimal product formulations and schedules to maximize impact and minimize cost and operational difficulties. ☐ Assist the country decision-making process, build an evidence base of country experience and methodology at the international level for each new vaccine and technology. ☐ Ensure that the long-term financial requirements from national governments and supporting partners are fully understood and committed to prior to the introduction of new vaccines.
Strategy 9: Ensure effective and sustainable introduction of new vaccines and technologies	☐ Integrate the introduction of each new vaccine into countries' multi-year sector-wide plans and provide a financial analysis. ☐ Ensure adequate training of health workers and vaccine managers at all levels and prepare the logistics and reporting systems. ☐ Produce appropriate information, education and communication materials to ensure good understanding of the benefits of new vaccines or technologies, and their acceptance by parents, communities and health workers. ☐ Ensure that within five years of introduction the coverage of the new vaccine reaches the same level of coverage as for other vaccines given at the same time. ☐ Expand surveillance of diseases that can be prevented by new vaccines, and strengthen laboratory capacity to monitor the impact of these new vaccines on disease patterns and programme operations.

GIVS strategies	Activities
Strategy 10: Promote research and development of vaccines against diseases of public health importance	■ Produce local evidence to influence and prioritize public and private investments in new vaccines and technologies. ■ Engage local public health authorities and research communities in defining research agendas relevant to countries which bear a disproportionate share of the disease burden. ■ Strengthen the capacity of developing countries to undertake the research and development of new vaccines and technologies, including conducting high quality clinical trials and post-licensure evaluations. ■ Generate geographically and epidemiologically representative clinical data on vaccine effectiveness and conduct demonstration projects of post-licensure evaluations of the impact of vaccination on child survival. ■ Engage the global research and development community, including vaccine manufacturers, in the design and production of new vaccines against infectious diseases of public health importance, especially in developing countries. ■ Research and develop evidence-based policies for immunization schedules and strategies as new vaccines and vaccine presentations (e.g., vaccine aerosols) and technologies are introduced.

Strategic Area III: Integrating immunization, other linked health interventions and surveillance in the health systems context

GIVS strategies	Activities
Strategy 11: Strengthen immunization programmes within the context of health	■ Through regular analysis of district-wide data, document key factors for the success and failure of immunization activities and share these findings with others involved in health systems development. ■ Participate actively in collective efforts to shape sector-wide policies and programmes, while preserving the central role of immunization in the context of sector-wide policies and programmes. ■ Use the experience gained in health systems development as an opportunity to position immunization services in a way that ensures the maximum benefit for all people.
Strategy 12: Improve management of human resources	■ Inventory human resource needs and determine how existing trained immunization personnel can best contribute their skills and experience to new immunization and health systems goals, and engage nongovernmental organizations and the private sector in the delivery of immunization. ■ Plan for and provide sufficient, adequately paid and trained human resources and match human and financial resources to actual programme needs. ■ Through improved and secure living and working conditions, training and incentives (including career advancement, improved salaries and family support), motivate health workers in inaccessible or insecure areas to reach all eligible populations. ■ Ensure that supportive supervision to these health workers is resourced, prioritized, reliably conducted and monitored.
Strategy 13: Assess and develop appropriate interventions for integration	■ Develop and field test potential joint interventions according to national and regional priorities to assess their feasibility, safety and potential impact on disease reduction, and document these findings. ■ Tailor integrated packages of interventions to local needs and feasibility and ensure that they are mutually supportive and designed to meet demand. ■ At the global level, develop standardized methods for monitoring and evaluating the efficiency, effectiveness and impact of combined interventions, and adapt them for use at the district and service-delivery level.

GIVS strategies	Activities
Strategy 14: Maximize the synergy from integrating interventions	■ Include joint interventions in multi-year and annual plans, ensuring the acceptance and participation of all stakeholders within the programmes, the district management teams and the community. ■ Formulate and implement as part of these plans, integrated training plans based on training needs assessments and appropriately developed training material. ■ Implement interventions jointly, choosing from fixed, outreach, mobile, Child Health Day and supplementary immunization activity approaches. Special emphasis should be placed on outreach and mobile teams in situations where they represent the best means of contact between hard-to-reach populations and health services. ■ Monitor and evaluate the incremental efficiency, effectiveness and impact of combined interventions and their means of delivery; apply these findings in order to continuously improve the combined intervention, increase the range of joint interventions, and contribute to long-term financial sustainability.
Strategy 15: Sustain the benefits of integrated interventions	■ Bridge different programmes in global agencies and within countries by formalizing a management structure that facilitates coordination and efficiency without disregarding programme-specific needs. ■ Establish joint financing, monitoring and evaluation functions. ■ Pool the resources needed to cover operational and other costs. ■ Remain attentive to community-perceived needs and provide quality information to secure sustained community support. ■ Advocate for further synergy and explore additional linkages.
Strategy 16: Strengthen monitoring of coverage and case-based surveillance	■ Expand the existing surveillance systems (such as polio and measles surveillance) in order to progress towards effective case-based surveillance for vaccine-preventable diseases, i.e., both existing vaccine-preventable diseases and diseases for which vaccines are anticipated. ■ Improve coverage monitoring of vaccines and other linked health interventions and the use of information at district and local levels through strengthening human resource capacity, monitoring the quality of data, improved tools for data compilation, feedback and supervision. ■ At the global level, develop and provide countries with new methodologies to estimate the burden of disease in order to obtain more accurate estimates of disease and to monitor vaccination coverage and programme performance towards achieving national, regional and global goals.

GIVS strategies	Activities
Strategy 17: Strengthen laboratory capacity through the creation of laboratory networks	■ Expand the existing laboratory networks (including the polio and measles laboratory network and other regional and local networks such as the Paediatric Bacterial Meningitis Network and the networks established by GAVI's Accelerated Development and Introduction Plans for pneumococcal and rotavirus vaccines) to include other priority diseases. ■ Assure the training, equipment, reagents and quality control procedures needed to sustain high quality diagnostics for all vaccine-preventable diseases and other priority diseases. ■ At the global level, develop new diagnostic tests, tools and procedures to improve both field-based and laboratory confirmation of diagnoses.
Strategy 18: Strengthen the management, analysis, interpretation, use and exchange of data at all levels	■ Contribute to the design of integrated management information systems and improve data management through regular training, monitoring and feedback at the local level. ■ Regularly review district indicators of performance, including risk status for vaccine-preventable diseases and use surveillance and monitoring data to advocate for improved access to and quality of immunization. ■ Contribute to the development of better tools (e.g., computer software) for monitoring coverage of vaccines and linked interventions, vaccine and logistics management, and disease surveillance to better support data entry, analysis, feedback, and utilization for programme management. ■ Monitor the quality and performance of coverage monitoring and surveillance systems through surveys, monitoring of performance indicators, data quality assessments, disease modelling and supportive supervision. ■ Collaborate with civil authorities in advocating for increased registration of births and deaths.

GIVS strategies	Activities
Strategy 19: Provide access to immunization services in complex humanitarian emergencies	■ Include immunization-related issues in rapid situation assessment of complex emergencies. ■ Incorporate immunization services in emergency preparedness plans and activities. ■ Re-establish immunization services in populations affected by complex emergencies and link these services to the rehabilitation of health systems. ■ Create global capacity to advise on appropriate immunization strategies in complex emergencies and natural disasters. ■ Include vaccine-preventable diseases in integrated surveillance and monitoring systems established in response to complex emergencies.

Strategic Area IV: Immunizing in a context of global interdependence	
GIVS strategies	**Activities**
Strategy 20: Ensure reliable global supply of affordable vaccines of assured quality	■ Ensure long-term forecasting for existing and new vaccines through close collaboration between international agencies, donors and vaccine manufacturers. ■ Develop global standards and methods for testing the quality, safety and efficacy of vaccines and other biologicals. ■ Promote the production of affordable vaccines of assured quality by vaccine manufacturers in developing and developed countries. ■ Promote the emergence of multiple manufacturers from industrialized and developing countries to provide an adequate supply of affordable vaccines of assured quality (both existing and new vaccines) and immunization materials.
Strategy 21: Ensure adequate and sustainable financing of national immunization systems	■ Strengthen national capacity for financial planning both within the immunization programme itself and the ministry of health as a whole. ■ Commit increased and sustained national budget allocations for vaccines on the basis of improved understanding of the value of vaccines in public health. ■ Encourage local and district level contributions to health services and immunization programmes through interaction with local businesses and interests. ■ Mobilize international solidarity to secure and sustain financing for immunization, including long-term commitments by existing public and private funding entities and new long-term financial mechanisms in support of the research, development, production and use of new vaccines. ■ Coordinate immunization financing through the Interagency Coordinating Committees to ensure adequate and appropriate donor support to national governments.
Strategy 22: Improve communication and dissemination of information	■ Develop new ways of using the globalized media, including the Internet, to build public awareness of the benefits of immunization. ■ Produce quality and timely information on the benefits of immunization and associated risks, and develop key messages to promote immunization according to national needs and priorities. ■ Through regional and global channels, document and systematically communicate the experience gained by countries that have added new vaccines and technologies.

GIVS strategies	Activities
Strategy 23: Define and recognize the roles, responsibilities and accountability of partners	■ Negotiate and define the roles and responsibilities of key immunization partners at the global level on a regular basis to ensure both accountability and efficient coordination. ■ Set global immunization goals jointly and in consultation with countries to ensure the full commitment of all parties. ■ Obtain global concurrence on policies, norms and standards for immunization and additional interventions. ■ Provide leadership in global advocacy and ensure that immunization remains high on the global health agenda by raising awareness of the importance and benefits of immunization among governments and donors and in the global community. ■ Develop and actively participate in regional and national partnership bodies (such as Interagency Coordinating Committees) to support implementation, provide ongoing technical assistance and monitor progress in countries. ■ Support epidemiological and operational research on vaccines and immunization.
Strategy 24: Include vaccines in global epidemic preparedness plans and measures	■ Develop global and country-specific epidemic preparedness and prevention plans relevant to specific diseases. ■ Develop and implement plans and funding for a stockpile of key vaccines for both epidemic control and pre-emptive campaigns. ■ Maintain an effective surveillance system linked to the Global Alert and Response Network enabling the appropriate and timely use of vaccines in the context of emerging or threatening epidemics, and share information globally. ■ Strengthen regulatory capacity to respond to urgent needs for epidemic preparedness and response.

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EXHIBIT C

"Can Needle–free Administration of Vaccines Become the Norm in Global Immunization?"
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If vaccines could be administered without needles and syringes ('sharps'), immunization practice would become safer, more accepted and more suitable for mass use. The author explores the status of technologies that could achieve this aim and the barriers that must be overcome for their implementation.

Can needle-free administration of vaccines become the norm in global immunization?

MYRON M. LEVINE

Vaccines constitute one of the most cost-effective preventive measures against illness and death from infectious disease. Even as modern biotechnology is generating vaccines against infections that were previously unpreventable, and is improving existing vaccines, other fundamental changes in vaccines and immunization are unfolding on the world scene. Increasingly, the vaccines received by children and adults in industrialized countries are distinct from those given in developing countries, as are their site of manufacture and popular perceptions of vaccine safety (Table 1). In developing countries, infections such as measles (which accounts for ~800,000 deaths annually) and pertussis remain killers, whereas in many industrialized countries, where vaccines have controlled these diseases, there is often greater concern over perceived adverse effects of vaccines than over the diseases themselves. Nevertheless, three fundamental themes remain in common worldwide: first, high immunization coverage of target populations generally must be attained for maximal public health impact; second, most current vaccines are administered parenterally using a needle and syringe; third, there is a broad recognition of the need to find ways to administer vaccines without the use of 'sharps' (that is, needles and syringes).

Why needle-free immunization is desirable

Concerns about the number of injections that must be given to infants and toddlers is driving the development of parenteral combination vaccines. In developed countries, immunization without needles or syringes would increase acceptability (and therefore compliance) and would enhance occupational safety for vaccinators and other health providers. This could be particularly critical in the future should it become necessary to immunize large populations rapidly *en masse* in the face of a pandemic influenza or bioterror emergency.

Needle-free immunization is even more critical for developing countries, where expanded immunization coverage and the addition of new vaccines could prevent millions of childhood deaths. Since the mid-1970s, the World Health Organization's Expanded Programme on Immunization (EPI) has recommended six basic vaccines for infants in developing countries: diphtheria and tetanus toxoids, whole-cell pertussis, bacillus Calmette-Guerin (BCG), and attenuated polio and measles; hepatitis B and *Haemophilus influenzae* type b (Hib) conjugate (where disease burden indicates) were recommended subsequently. Developing countries are also increasingly using mass immunization campaigns to drive measles from communities and to curtail meningococcal epidemics. The Global Alliance for Vaccines and Immunization (GAVI) and its associated Vaccine Fund (which was initially capitalized with $750 million from the Bill and Melinda Gates Foundation and later expanded by other donations to ~$1.2 billion) are addressing the long delay in the introduction of lifesaving vaccines between industrialized versus developing countries.

In developing countries, delivery of immunization would be more efficient and economical if all vaccines were temperature stable, required less than three doses to immunize, and could be administered without needles. However, except for the oral polio vaccine, all EPI vaccines are now given using needle and syringe. This is problematic because in developing countries injection safety is a notorious problem[1,2]: improper practices involving nonsterile needles and syringes (often reused from one person to another) cause abscesses and transmit blood-borne pathogens (such as hepatitis B and C and HIV)[2]. Single-use 'auto-disable syringes' provide a partial solution by preventing reuse, but generate infectious waste that must be properly handled lest it endanger bystanders. Although parenteral vaccination accounts for only a fraction of the needles used by health workers, immunization is held to a higher standard than other uses of needles because it involves healthy individuals.

This commentary reviews technologies for needle-free administration of vaccines across mucosal surfaces or through the skin, and considers their practicality for use in developing countries with current and future vaccines and suitability for low (for example, small health centers) and high workloads (for example, mass campaigns), as well as the practical, logistical and economic barriers to development and deployment.

Vaccines delivered through mucosal surfaces

Among the possible mucosal routes (including oral, nasal, rectal, conjunctival and vaginal) for immunization of humans, oral and nasal are practical for all ages and both genders (Fig. 1)[3]. Specialized microfold (M) cells overlying mucosa-associated lymphoid tissues in the intestine and nose constitute effective portals by which vaccine antigens reach underlying inductive sites for immune responses[4]. Properly formulated, mucosally administered vaccines can stimulate any relevant type of immune response: secretory IgA (S-IgA), serum IgG–neutralizing antibodies (against toxins and viruses) and cell-mediated responses (lymphocyte proliferation, cytokine production and $CD8^+$ cytotoxic lymphocyte activity). Because they elicit S-IgA, mucosal vaccines are attractive for use against pathogens that cause mucosal infection or invade through the mucosa. Some mucosal vaccines, such as Ty21a live oral typhoid vaccine, stimulate long-term protection lasting up to 7 years[5].

Oral vaccines. Oral polio vaccine sets the standard for ease of administration to individuals of any age. The overall experience with licensed oral vaccines, including Ty21a (ref. 5), live cholera vaccine strain CVD 103-HgR (ref. 6) and nonliving cholera vaccines[7,8], has been positive, although some problems have appeared. In the United States, post-licensure sur-

Table 1 Diverging immunization realities

	Industrialized countries	Developing countries
Infant combination vaccines	Acellular pertussis-based	Whole-cell pertussis-based
Measles vaccine	Trivalent MMR	Monovalent measles
Polio vaccine	Inactivated parenteral	Live oral
BCG	Uncommon	Routine
Varicella, pneumococcal conjugate	Increasingly common	Not introduced yet
Immunization schedule extends to year 2 of life	Routine	Uncommon
Source of vaccine	Industrialized country manufacturers	Mostly developing country manufacturers
Use of multidose vials	Minority of vaccine used	Majority of vaccine used
Public perception	Concerns over vaccine safety	Fear of disease

In contrast to the situation summarized here, as recently as the mid-1980s there was little difference in the array of vaccines given to infants in the industrialized and developing world. In addition, a notable proportion of vaccines procured by UNICEF for developing countries were from manufacturers in industrialized countries (mainly European). BCG, bacillus Calmette-Guerin; MMR, measles-mumps-rubella.

veillance detected an uncommon association between tetravalent reassortant rhesus rotavirus vaccine and intestinal intussusception[9], resulting in its withdrawal from the market. New rotavirus, and perhaps other oral infant vaccines, must address the risk of intussusception through large clinical trials before licensure. It has also been observed that some live oral vaccines are less immunogenic in individuals in developing countries than in industrialized states[10–12]. Contributing influences include small-bowel bacterial overgrowth[13], intestinal helminths[14] and competing enteric viruses.

Unfortunately, there is little clinical experience with platform technologies that might allow existing EPI vaccines to be administered orally or allow development of alternative oral EPI vaccines[3]. These include bacterial and viral live vectors expressing foreign antigens, DNA vaccines administered directly or by means of bacterial vectors[15], transgenic plant 'edible vaccines', and various nonliving antigen delivery systems including liposomes, proteosomes and polylactide/polyglycolide microspheres. Clinical trials with these technologies have yielded mixed results[16–18], some of which are promising[16,17].

Nasal vaccines. Live, cold-adapted trivalent influenza vaccine, administered by a single-use spray device that painlessly delivers vaccine to the nasal mucosa (Fig. 1), is under consideration for licensure by the US Food and Drug Administration (FDA), on the basis of its safety, immunogenicity and efficacy[19,20].

Researchers are seeking well-tolerated adjuvants to enhance immunological responses to nonliving vaccines administered through mucosal surfaces. Cholera toxin (CT) and heat-labile enterotoxin (LT) of enterotoxigenic *Escherichia coli* are powerful adjuvants that enhance local S-IgA and serum antibody responses to coadministered soluble or particulate antigens. Although they are unacceptable as human oral adjuvants, in that as little as 5.0 μg causes severe diarrhea[21], they have been explored for nasal use. LT adjuvant was incorporated in a nasal, nonliving influenza vaccine used in Europe[22]. Disappointingly, however, postlicensure surveillance identified a possible association with cases of Bell's palsy, leading to withdrawal of the vaccine from the market. To increase safety, mutant LT and CT molecules have been modified to reduce toxicity but retain adjuvanticity for antigens coadministered intranasally[23,24]. Safety concerns remain, because in some species ganglioside-binding properties of mutant LT and CT allow uptake by nasal olfactory nerve fibers and retrograde transport to the olfactory lobes of the brain[25]. Because it is not known whether this occurs in humans and what consequences might result, clinical trials using mutant toxins intranasally must proceed with due caution and watch carefully for adverse effects.

Safer intranasal adjuvants are needed. An intriguing one is CTA1-DD, which links the enzymatically active subunit A of CT to an Ig receptor-binding peptide[26], thereby targeting the immune system's B cells[26]. Theoretically, adjuvants like CTA1-DD could be coadministered intranasally with existing diphtheria-pertussis-tetanus (DTP), hepatitis B (HBV) and Hib conjugate vaccines. The effectiveness of these promising nasal vaccination strategies must be documented in infants in developing countries, in whom upper respiratory infections and nasal discharge are highly prevalent.

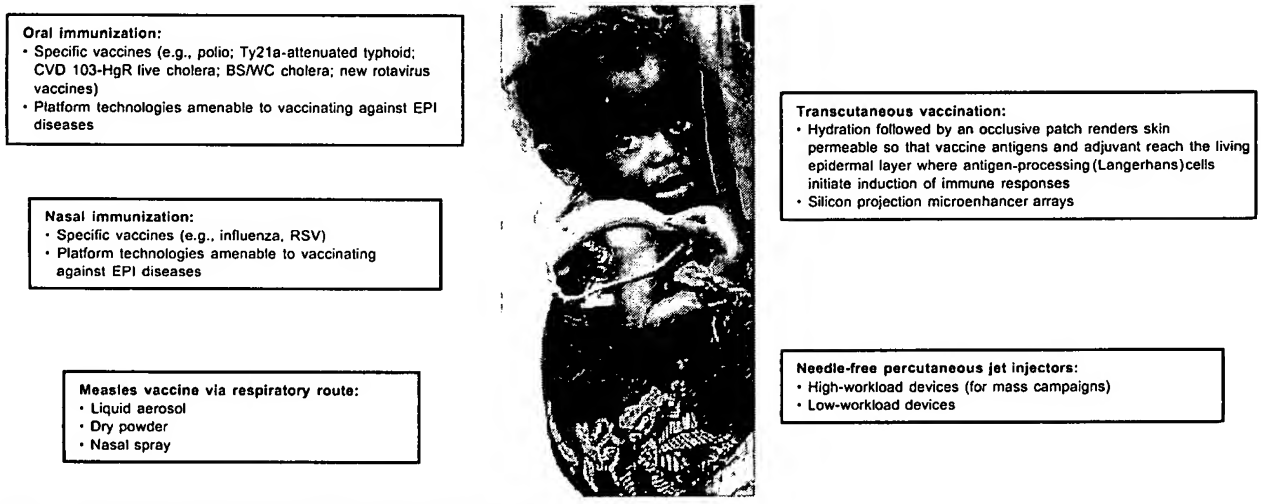

Fig. 1 Promising needle-free vaccination technologies and strategies.

Table 2 Needle-free vaccine technologies versus needle and syringe

Needle-free strategy	Ease of use for health worker	Use with existing 'off-the-shelf' vaccines	Suitability for low workloads[a]	Suitability for mass immunization campaigns	Generates dangerous infectious waste	Remaining clinical development hurdles	Production and supply issues	Financial barrier[b]
Oral								
Adjuvant	5+	Yes	5+	5+	No	4+	3+	3+
Platform technologies[c]	5+	No	5+	5+	No	4+	4+	4+
Nasal and aerosol								
Adjuvant	4+	Yes	5+	4+	No	4+	3+	3+
Platform technologies[c]	4+	No	4+	4+	No	4+	4+	4+
Aerosol measles	3+	Yes	2+	4+	No	3+	3+[d]	2+
Transcutaneous								
Simple patch	3+	Yes	4+	3+	No	4+	3+	3+
Specially formulated patch	4+	No	5+	4+	No	4+	4+	4+
Jet injectors								
Experimental multiple-use nozzle	2+	Yes	2+	5+	No	5+	4+	4+
Single-use nozzle	3+	Yes	4+	3+	No	2+ (or none)	3+	4+
Single-use nozzle with 'universal' cartridge	3+	Yes	4+	4+	No	3+	4+	5+
Current 'sharps' technology								
Auto-disable needle and syringe	3+	Yes	4+	3+	Yes	None	2+	1+

[a]Small health centers and outreach programs. [b]Includes the estimated costs of clinical development leading to licensure, process development, large-scale production and widespread implementation of a needle-free technology to administer current or alternative EPI vaccines against currently targeted diseases. [c]For example, DNA vaccines, live vectors, microspheres, proteosomes, liposomes, transgenic plants. [d] Related to developing a suitable rapid, robust portable aerosol device.

Aerosol measles vaccine. Mass immunization campaigns in Latin America and pilot campaigns in southern Africa with parenteral measles vaccine have decreased measles incidence and mortality. Nevertheless, such campaigns would be simpler and safer if measles vaccine could be administered without needles and by aerosol (creating small particles that reach the lung), which is known to be immunogenic and efficacious[27,28]. Whereas early aerosol measles vaccine devices had limitations in rapidity of use, portability or robustness, there are now under evaluation devices that are both simpler and more compact.

Vaccines delivered through the skin without needle and syringe

Jet injectors. Jet injectors are needle-free instruments that propel liquid (live or nonliving) vaccine through a minute orifice under high pressure percutaneously to the dermis, subcutaneous tissue or muscle (Fig. 1). They induce immune responses comparable to vaccine injected by needle and syringe, albeit accompanied by somewhat higher rates of local reactions. From the 1950s through the 1980s, multiple-use nozzle jet injectors were widely used in mass immunizations against such diseases as smallpox, measles and yellow fever in less developed countries[29]. Multiple (up to 50)-dose vials allowed these devices to vaccinate 600–1000 individuals per hour using the same dose chamber (replenished automatically from the multiple-dose vial after each injection), fluid path and nozzle on consecutive individuals. In the mid-1980s it was recognized that multiple-use nozzle jet injectors could, albeit rarely, transmit blood-borne infections such as hepatitis B (ref. 30). The global emergence of HIV led to their discontinuation.

The 2001 anthrax emergency in the United States has prompted public health authorities to consider how they might conduct mass immunization campaigns if confronted by a bioterror event. This threat has kindled interest in new high-workload devices such as a multiple-use nozzle jet injector that incorporates a disposable cap to reduce the risk of splashback of blood or serum onto the nozzle after injection and injectors that use disposable cartridges that self-disable after use. Should clinical trials document their safety, these instruments could be used in developing countries in mass campaigns against, for example, measles and meningitis.

For administering vaccines in lower-workload situations, there are also available single-dose jet injectors that use disposable cartridges and nozzles for each individual to avoid cross-contamination[31]. Some (such as the Biojector 2000) are presently used to vaccinate in physicians' offices and clinics in industrialized countries to overcome aversion to needles and to avoid needle-stick injuries[31,32]. These devices are not yet affordable for developing countries. One limitation of these instruments is that the vaccine must be transferred from its vial into the cartridge using a needle or special adaptor, a potential complication in developing-country settings[33]. Although this intermediate step is advantageous for reconstituting lyophilized vaccines, it could be avoided if manufacturers prefilled vac-

cines directly into universal standard single-dose cartridges that could fit a variety of injectors. One experimental system, the Imule cartridge[34], which inserted into a hand-wound spring-powered injector (Mini-Imojet)[34], produced encouraging results in adults and infants in industrialized and developing countries[34]. Needle-free devices have also successfully delivered DNA vaccines in clinical trials[35].

Transcutaneous administration of vaccines. Recognition that skin is a highly competent immunological organ replete with dendritic antigen-presenting (Langerhans) cells has led to attempts at 'transcutaneous immunization' (Fig. 1). Hydration followed by application of an adhesive patch makes skin permeable so that vaccine antigens and adjuvant can reach the living epidermis, where Langerhans cells are attracted, take up antigen and initiate induction of immune responses[36]. Results of preliminary clinical trials in which patches with *E. coli* antigen and LT were applied to the skin of healthy adults were encouraging[37]. More practical dry-patch formulations impregnated with various antigens and adjuvant are being prepared for clinical trials. This highly flexible technology holds much potential.

In preclinical studies, DNA vaccines have been administered transcutaneously using various techniques, including an instrument bearing arrays of micron-scale silicon projections (microenhancer arrays or MEAs) that breach the stratum corneum, allowing vaccine to reach the epidermis and to elicit strong immune responses[38]. These promising results are prompting clinical trials.

Feasibility, opportunity and barriers

New oral (for example, rotavirus, *Shigella*, typhoid) and intranasal (for example, respiratory syncytial virus (RSV), parainfluenza) and perhaps also transcutaneous vaccines against specific diseases will surely become licensed during this decade. But what is the likelihood that needle-free vaccination can completely replace the routine vaccines that are currently administered with needles, particularly in developing countries? Accelerated development and implementation of needle-free vaccination technologies are impeded by three formidable barriers: (i) insufficient clinical data, (ii) financial ramifications and (iii) global diversity in immunization practices. It is improbable that alternative EPI vaccines based on platform technologies such as live vectors and DNA vaccines would garner investment to complete clinical development and create manufacturing capacity. This is because the path to licensure of new vaccines, which is based on demonstrating their safety, efficacy and consistency of manufacture, is expensive, time-consuming and very risky. The result is that few succeed[39] (Table 2). It is more likely that investment in platform technologies will be attracted to support the development of vaccines against previously unpreventable infections[39].

Obtaining regulatory approval to administer existing vaccines by alternative routes should be faster and cheaper, particularly when immunological correlates of protection exist. Thus, aerosol or intranasal administration of measles vaccine (either alone, with rubella or as trivalent measles-mumps-rubella) might attract investment, as might the switching of administration routes of existing parenteral EPI vaccines to intranasal or transcutaneous (Table 2). Nevertheless, the hurdle faced by generic strategies to document safety and immunogenicity equivalence must not be underestimated.

Arguably, the greatest likelihood of biological success for non-needle delivery of existing (and future) parenteral vaccines could be by improved jet injectors. In industrialized countries, preparations to immunize populations *en masse* against pandemic influenza and bioterror threats, as well as cultural aversion to needles, are driving interest in jet injectors. However, products geared to populations in the industrialized world will probably be too expensive for developing world needs. If jet injectors do come into wide use, it will further accentuate the divergence in the realities of immunization in the industrialized versus the developing world (Table 1).

Regulatory agencies in industrialized countries set extremely high standards with respect to vaccine safety, often with far-reaching consequences. Following FDA recommendations that thimerosal, which contains ethyl mercury and is used in vaccine production as a bacteriostatic preservative in multidose vials (as well as for other purposes), be removed because of concerns about possible risk of mercury exposure for immunized infants[40], US and European manufacturers are moving to produce, exclusively, single-dose vials.

For the foreseeable future, vaccine manufacturers in developing countries will continue to produce multidose vials containing thimerosal, because a switch to single-dose presentation will require large investments to alter production lines and would increase cost per dose and volume to be handled by the cold chain (refrigerators and transport containers that control temperature thereby protecting live vaccine from high temperature and protein vaccine from freezing) (Table 1). On the positive side, single doses diminish vaccine wastage, a critical concern for expensive vaccines and settings (for example, outreach) where wastage is high. Given the major changes in production already underway, some may contend this is a propitious moment for manufacturers in industrialized and developing countries alike to consider filling their vaccines into consensus single-dose universal cartridges that could be administered by means of an array of commissioned single-use-nozzle, auto-destruct needle-free injectors. Because such cartridges can be markedly smaller than single-dose vaccine vials, cold-chain storage requirements would be less. In reality, the enormous investment needed to modify production lines globally and to provide jet injectors is likely to be prohibitive in comparison with the benefits gained. Moreover, such a change could not proceed unless agreed to by all stakeholders, including countries, manufacturers and international agencies.

This author's long-term enthusiasm for investigating ways to immunize without needles remains unabated, and biotechnology continues to expand the options. Nevertheless, economic constraints, logistical and safety concerns, and diverging immunization practices make it unlikely that in the foreseeable future needle-free vaccination can completely replace the use of needles worldwide. Yet optimism is still warranted for the vision that, in the more distant future, all vaccines will be administered without needles and syringes.

Note: The opinions expressed in this commentary are personal views of the author and do not represent institutional or agency endorsements.

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EXHIBIT D

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Vaccines for children: policies, politics and poverty

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Abstract

The dawn of the 21st century ushered in spectacular advances in vaccine production technology. However, the benefits of these developments have been largely confined to the world's most affluent and least afflicted. Of the 14 million deaths that occur world-wide in children aged less than 5 years, over 95% of these occur in developing countries and at least 70% are caused by infections for which vaccines are already available in other countries.

While impoverished countries do not have a right to be assisted with the provision of funds or vaccines by affluent developed countries, an initiative for the global eradication of a vaccine preventable disease, requires a global effort. Assisting developing countries to achieve such goals should be a high priority for wealthy nations, even if only to protect their own populations. With improved international travel, not only can newly emerging diseases spread across the globe, but pathogens eliminated from one population can be re-imported by travellers or immigrants.

In contrast, the recent decline in acceptance of immunisation programmes in developed countries are secondary to strong anti-vaccine movements attributing unproven adverse reactions to vaccines, placing these life-saving vaccines into disrepute. A fertile ground for propagation of these ideologies is created by parents who in their lifetime may not have seen a child killed or maimed from bacterial meningitis or measles and therefore have little understanding of the risk–benefit of vaccination.

The development and deployment of vaccines must be a global effort as are the treaties for global disarmament for weapons of mass destruction.

Keywords: Vaccines; Poverty; Politics

1. Introduction

Of the 14 million deaths that occur world-wide in children aged less than 5 years, over 95% of these occur in developing countries and at least 70% are caused by vaccine preventable infections [1,2]. Despite these staggering statistics, in most developing countries a significant proportion of the health budget allocation is spent on curative health with considerably less funds and resources allocated to preventive health. Understanding how much of this ill-management is due to misplaced priorities or ignorance is crucial for defining strategies for ensuring a sustained mechanism for the supply and use of vaccines in the most afflicted and impoverished parts of the world.

Several international agencies, non-governmental organisations and philanthropic agents continue to make enormous contributions to the procurement of vaccines for use in poor countries. However, several countries that have benefited from these schemes have very little or no appreciation of the value of this assistance. This in part may be due to lack of appreciation of the burden of disease attributable to infection that is being prevented by these vaccines.

In contrast, most developed countries are not limited by funds. The epidemiology of majority of infectious diseases is well characterised and health needs have been appropriately prioritised. Vaccines are made available to the general public. Over the years, there has been a significant decline in incidence of most vaccine preventable diseases. The current epidemiology of infectious diseases in these two 'worlds', has a significant impact on the perception of the value of vaccines.

This manuscript highlights some of the recent key progress in making vaccines available globally, the remaining obstacles with the development of needed vaccines, deployment of existing vaccines and offers suggestions for strategies that may overcome these barriers.

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1.1. An alliance

The recent effort in bringing together traditional and new partners from the private and public sectors with a strategic objective of making vaccines more readily available to the world's children, particularly those in impoverished regions of the world, culminated in the birth of the Global Alliance of Vaccines and Immunization (GAVI). This development has given the problem of vaccine supply to impoverished countries a major leap [3]. Through this effort many countries have received, and many more will receive assistance in procuring much needed life-saving vaccines for a limited number of years after which they would be expected to sponsor the procurement of their own vaccines. This is a great start for many countries but critical issues relating to sustainability after the honeymoon period has not received much attention. While these efforts deserve high commendation, issues critical to financial sustainability deserve careful attention. To avert the syndrome of donor dependency, recipients of these schemes should establish a genuine need, be convinced that they are not been exploited and that these vaccines are not only cost saving but also live-saving.

1.2. Donor dependency

The immunisation programmes in several developing countries are currently supported wholly or in part by donations from philanthropic organisations from developed countries. While these efforts deserve high commendation, such donations are not infinite, and will eventually expire or be routed to new priorities leaving the recipient countries without support. It is pertinent therefore that arrangements are in place for sustainability of these services at the end of the donor term.

It is often the case that poor countries become dependent on donor resources with little or no effort to generate indigenous sources for funding these services and this state of complete dependency only comes to light after the financial support has been withdrawn.

A continued critical appraisal of the political commitment to immunisation services and the utilisation of these services by the public in addition to continued advocacy will be useful in ensuring commitment to sustaining improved services.

2. Impediments to the development of new and deployment of existing vaccines

There are already several vaccines in routine use in developed countries that have proven efficacy and can potentially save thousands of lives in developing countries but these have been under-utilised (Table 1). The reasons for the poor utilisation of these vaccines include lack of disease burden data, poverty and misplaced healthcare priorities.

Table 1
Licensed but poorly utilised vaccines

Haemophilus influenzae type b conjugate
Pneumococcal conjugate
Typhoid Ty21a
Hepatitis A virus inactivated
Varicella
Measles, mumps, rubella (MMR)
Japanese encephalitis
Yellow fever

2.1. Disease burden

The primary motivation for the development or deployment of a new vaccine is the established burden of disease, and in some cases the rising cost of care, consequent to pathogens which have developed multi-antimicrobial resistance. Multi-antimicrobial resistance is not often a problem in most developing countries, as the choice of antimicrobials is often limited and extensive disease progression before presentation to a health care provider remains the norm. Unfortunately, in these settings, morbidity and mortality is caused by a myriad of infectious pathogens and the facilities to make diagnostic definitions for the determination of pathogen-specific disease burden is often lacking. Thus, specific disease prevalence may be high but as this is not recognised, the need for a specific and appropriate intervention such as vaccination is not appropriated.

In addition to disease burden, the differences in epidemiology of infectious diseases in developing and developed countries also influence the development of vaccines. On a global scale, certain infectious diseases are entirely or largely limited to developing (mostly tropical) countries. Prominent among these are parasitic diseases including malaria, leishmaniasis and schistosomiasis, for which the global burdens are huge. The proportion of resources available for vaccine development against these infections, however, does not reflect their global public health importance. In contrast, although the toll of AIDS is greatest in developing countries, it is common in both developed and developing countries and has therefore made it to the priority list for new vaccine development. For such infections, government institutions, the vaccine industry and philanthropic foundations make huge efforts to develop vaccines.

2.2. Manufacturer's priority

The bulk of global vaccine supply originates from manufacturers based in developed countries. As with any commercial organisation, these companies are profit driven and efforts are directed at the development of vaccines that have a market largely in developed affluent countries. Consequently, diseases that are confined to developing, poor countries are not a priority for these manufacturers. A typical example is the development of the vaccines against meningococcal disease. Meningococcal disease may be caused by

one of several groups of *Neisseria meningitidis*. Of the prevalent types, group A disease is predominant in developing countries with attack rates as high as 100 per 100,000 and case fatality rates in excess of 10% [4]. In 1996, an epidemic that swept through West Africa recorded over 200,000 cases. In northern Nigeria alone, there were 109,580 cases and 11,717 deaths recorded, giving a case fatality rate of 10.7% overall [5]. In real life, fatalities would be in excess of these figures as a significant number of cases are often unable to make it to a health facility due to access or transportation problems. In contrast, group C disease is prevalent in developed countries but the attack rate in these settings is about two log scales less than that of group A disease in developing countries. The availability of improved basic medical care in these communities also contributes significantly to the lower case-specific mortality [6,7]. Almost a decade ago, a conjugate vaccine against serogroup A was tested with excellent initial antibody response but levels declined after a few years [8]. Further development was not pursued because there was no promising market for the manufacturers. However, the meningococcal C vaccine has been extensively researched and is currently licensed and routinely used in the UK. There was no formal efficacy trial of the meningococcal C conjugate vaccine before its licensure and implementation in the UK but the impact on the incidence of meningococcal disease in the UK has been overwhelming [9,10]. Only recently has there been a further clinical re-evaluation of a conjugate vaccine with the serogroup A component and attempts at mass production and licensure [11–13].

2.3. Deploying existing vaccines

In developing countries, the reasons for under-utilisation of existing licensed vaccines are diverse and complex (Table 2). They vary from the obvious lack of substantiated or perceived need to lack of funds for procurement. In many developing countries attention needs to be paid to the improvement of existing infrastructure and vaccine delivery systems.

The market driven vaccine development has implications for the deployment of existing vaccines used in developing countries. The development of combination vaccines, for example, the pentavalent (hepatitis B, diphtheria, tetanus, acellular pertussis and *Haemophilus influenzae* type b (Hib) vaccine combination), was developed primarily for use in developed countries. This development has been a blessing in disguise for some countries who have recently been unable to procure the DTwP–Hep-B vaccine and have been providentially offered the DTaP–Hep-B Hib combination instead, in most instances in Africa, through the Global Alliance for Vaccines and Immunisation. While this serendipitous development will save many lives the recipient countries may not have been given an opportunity to establish the need for such combination vaccines and this could be detrimental to making the required financial and resource commitment for sustaining the use of such vaccine combinations.

Table 2
Impediments to improved utilisation of licensed vaccines or adoption of new vaccines

Lack of disease burden data
Poor perception of disease burden
Poor perception of the value of vaccine
Dilapidated cold chain infrastructure
Poor transportation systems
Poor vaccine management
Finance
Lack of informed and well-resourced local ethics committees
Lack of economic and /or political will
Misplaced priorities

2.4. Strengthening existing cold chain infrastructure

The major focus of most national immunisation programmes is usually vaccine procurement. This is however merely one aspect of this complex service. For an effective delivery of immunisation services there is a need to address all those factors in the health sector that impinge in anyway on the ultimate goal of raising vaccine coverage rates as to impact on disease burden and child survival. Such an investment would need to consider several critical areas. The infrastructure for storage, distribution and delivery of vaccines in most developing countries, particularly in sub-Saharan Africa are in need of major overhaul. There has been no formal system in place for continuous evaluation and replacement of refrigerators and solar panels. Consequently, there are frequent breaks in the cold chain and as this often goes unnoticed, may be responsible for vaccine failures and loss of public confidence in vaccines in some settings. The strengthening of the existing cold chain infrastructure, a costly investment, is a major challenge for developing countries and this should be an important component of assisted schemes in strengthening the immunisation programs in developing countries.

2.5. Provision of transport for the delivery of vaccines

Most developing countries do not have motorable road network systems to all parts of the country. In some settings, certain areas may only be accessible for limited periods of the year. The provision of reliable transportation is necessary to reach more vulnerable groups that are most at risk. While improving the road network systems in countries is certainly not within the remit of most international agencies and charity organisations involved with vaccine procurement, high level advocacy or incentive driven schemes may jolt governments into rearranging their priorities. Other creative mechanisms to address this constraint such as the use of two-wheel means of transportation need to be considered.

Decentralization of immunisation services is another option that would be worth exploring but only when there is a robust storage and delivery system or vaccines that are less dependent on the cold chain. The privatisation of

maintenance services, increasingly being assumed in some countries, may well prove beneficial in improving the maintenance and therefore the lifetime of vehicles.

2.6. Improved management of vaccines

Current methods for evaluating vaccine coverage which utilises cluster-sampling technique, does not reflect true regional or district coverage and does not provide objectivity, since this is often conducted by the service providers. Renewed focus and assistance should be provided to ensure the implementation of managerial practices that improve the availability of routine information to guide the planning, monitoring and evaluation of the service. These systems would also benefit from objective, seroepidemiological tools that can be used by the community or an external auditor to validate data collected from interview surveys. An example of this is the development of an immunological assay for the determination of immunity to tetanus or polio from oral fluid [14]. This assay could be used as a surrogate for measles or tetanus immunisation coverage. This approach warrants further development and field-testing.

In several regions immunisation coverage is low, in part because the drop out rate is high and there are no systems in place to facilitate the tracking of children who have not completed the course of immunisation. The record keeping system is very fragile, and is not subject-linked. Poor stock management at central and district levels often bedevil immunisation services. Computerised management systems have been suggested and may be appropriate in some settings. However, since in most developing country settings, literacy rates are low, electricity is unavailable and geographic co-ordinates are difficult to define or unreliable, computerised databases will have to be flexible and perhaps region specific.

Inadequate staffing often compromise the management of immunisation services and staff may be less well-motivated compared to other health sectors. To some extent with increasing prioritisation and funding of the national immunisation programmes, morale may improve.

2.7. Financing vaccine procurement

There are currently many new innovative funding mechanisms and financial assistance either in the form of loans or donations for the procurement of existing and new vaccines for developing countries. However, a critical issue that is yet to be addressed is the long-term sustainability of these schemes. This will always remain a key element of any immunisation programme in order to ensure uninterrupted supplies. The various schemes available provide funds or vaccines for a limited period, after which the recipient country is expected to assume full responsibility for funding of vaccines. Unfortunately, some of these mechanisms are associated with deals related to the procurement of vaccines that may not be in the long-term economic interest of the recipient. Availing the recipient country an opportunity to develop and maintain the capacity for conducting disease surveillance and establishing disease burden, before and after introducing a new vaccine, is crucial to motivating the economic and political commitment.

2.8. Economic and political obstacles

As with most issues with multinational involvement, there are economic and political hurdles in the way of immunisation services. These include limited resources, political instability, the high cost of vaccines, competing priorities and national pride, an important factor that is often overlooked by multinationals and International agencies. These agents in general have the facts, appropriate recommendation and funds to assist. Nevertheless, impoverished countries with enormous infectious disease burden, although in need of external aid, still want to maintain their identity and national pride. There is often an unspoken fear of dependence on the industrialised countries, suspicion of true motives, fear of exploitation and a strong desire for equity and national autonomy. These complex issues with the high cost of vaccines and competing priorities translate into problems of access and affordability, low priority for immunisation programmes and resistance to innovations in many settings. Unfortunately, not much is currently been done to address these sensitive issues.

3. Advocacy

Advocacy for vaccines or vaccine use in this context, connotes one or a combination of activities designed to:

(a) influence policy and decision makers;
(b) transform public perception and attitudes;
(c) modify behaviour;
(d) mobilise human and financial resources;

with the ultimate aim of improving availability and utilisation of vaccines.

Advocacy efforts to increase the use of a new vaccine or to improve the uptake of an existing vaccine can be generated from two sources. This may arise primarily from the recipient country, when the request for the use of the vaccine arises from the recipient country either as a perceived need or following the demonstration of a high disease burden, as was the case with the adoption of the meningococcal C conjugate vaccine in the UK. This is generally the source of advocacy in developed countries that have strong epidemiological services that can measure the burden of disease and can readily assess the value of a potential vaccine intervention. In contrast, advocacy may come from an external source, such as a philanthropic organisation that perceives the disease burden in a given setting for which there is an established efficacious vaccine.

Internal advocacy may offer a better sense of ownership of immunisation services to recipient countries and may also make it easier to rationalise programmes to the local consumers. Regardless of the source of advocacy, establishing the burden of disease, acceptability of the vaccine to the population are critical components of program sustainability. A formal demonstration of efficacy may in fact not be required, if there is a perceived high burden of disease with respect to morbidity and mortality and there is some immunogenicity data, as was demonstrated by the recent implementation of meningococcal C conjugate vaccine in the UK.

In both developing and developed countries, understanding the social and behavioural factors that influence the use of vaccines and immunisation services may have been neglected for too long with deleterious consequences. In general, because diseases with epidemic potential often carry a perceived risk that is greater than the actual hazard that they pose, the public readily accepts vaccines for such diseases. Thus public perception of risk is important as this may also adversely affect uptake of a vaccine, as has been the case with the MMR vaccine in the UK [15,16]. It is therefore crucial that the public are well informed through formal and informal advocacy programmes and public perception of vaccines is optimally utilised for the rational use of immunisation services.

In developing countries, the situation is potentially much more volatile as the prevalence of illiteracy and the absence of formal individual or public education could lead to rapid dissemination of rumours or misinformation. This situation is further compounded by the fact that in addition to the weak disease surveillance systems, systematic recording of vaccine related adverse reactions is ad hoc and virtually impossible in most settings. This makes accepting or rejecting claims of vaccine induced adverse reactions impossible to reject or accept. For instance, 2 years ago there was an observation from Guinea Bissau in West Africa suggesting that the diphtheria, pertussis, tetanus vaccine was associated with increased mortality in children [17]. While there are no convincing scientific explanations for why this association may occur, it has generated some concern in the scientific community. Fortunately, this preliminary observation has not been widely publicised in West Africa and this offers a window of opportunity for urgent verification of this observation. In any setting there is a need for continuous surveillance and public enlightenment about the benefit of immunisation services so that public confidence is retained.

3.1. Community involvement in national immunisation programmes

In developing countries, there is an urgent need to create awareness about the importance of vaccines as a major intervention in improving child survival. The level of awareness needs to be heightened such that communities are so well informed that they can demand this service of their government or initiate it through self-help projects. A heightened level of awareness may well serve as a catalyst to compel governments to allocate additional resources for national immunisation programmes. In developing countries, due to illiteracy, societal and cultural values, particularly, amongst women there have been misconceptions about the value of vaccines. There has been lack of awareness, false perceptions and irrational fears about vaccines. Widespread beliefs and prejudices of the lay public sometimes manipulated by mass media can greatly influence vaccine coverage and it should be possible to harness this to positively impact the value of vaccination. These misconceptions should be tackled with a structured approach that targets specifically but not exclusively, the opinion leaders in the communities, women, school children and policy makers. The providers of this service would also benefit from continuous education and refresher training sessions. These training programs will be enormously strengthened by constant review of surveillance data on vaccine preventable diseases.

3.2. Services in developed countries

In industrialised countries the problems are, in contrast, not related to illiteracy and poverty but largely, misconception about risks attributable to vaccines. Most adults living in developed countries, where the prevalence of infectious diseases are very low, have not experienced or witnessed the potential havoc that can be wrecked by vaccine preventable infections. Following the outstanding success of the *Haemophilus influenzae* type b (Hib) conjugate vaccines, many doctors are now graduating from postgraduate medical training without ever seeing a case of meningitis or epiglottitis caused by this bacterium. Thus, there is a danger that with time, the population will forget the benefits of this vaccine and dwell on the trivial and self-limiting adverse effects of vaccination such as a sore limb, irritability or low-grade fever. The MMR vaccine has received adverse publicity through the lay and scientific media in the UK, with deleterious consequences. Such unfortunate misinformation can be prevented, or at least their impact alleviated, by more concerted and structured information delivery system by the Health Department to the public.

4. Competing priorities and perceptions

Formulating vaccine policy is a complex process of balancing multiple risks and benefits. However, the variables in this complex equation differ considerably between developing and developed countries.

4.1. Calculating risk–benefit ratios

Since the early 1930s, thimerosal, an inorganic mercurial compound, has been used as an effective preservative in numerous medical and non-medical products, including multi-dose vaccine vials. Exposure to mercury during critical

stages of development may be associated with neurological disorders in children, such as attention deficit disorder, learning difficulties, and speech delays [18]. An experimental model was used to predict hair mercury concentration resulting from childhood immunisations and the estimated concentration was cause for concern [19]. Based on these findings, regulatory bodies including the FDA in several developed countries have recommended the withdrawal of thimerosal from all childhood vaccines [20,21]. Although the clinical consequences of thimerosal in childhood vaccines remain contentious [21], multi-dose vials are now no longer in use in these countries. It is likely that such a safety recommendation will be requested for all vaccines, globally in the near future. While the withdrawal of thimerosal from vaccine products is a prudent precautionary measure, this preservative has been in use for several decades without documented clinical toxicity. Since it would currently be impossible to ethically justify a placebo controlled trial to demonstrate the effect of thimerosal in childhood vaccines on neurodevelopment, some scientific consideration should have been made for the evaluation of a reduced thimerosal concentration in these vaccines or an evaluation of alternative preservatives. This recommendation may have little or modest implications for the affluent consumers in developed countries but it may have enormous operational and financial implications for the immunisation delivery services in developing countries. Procurement of single dose vaccines is likely to be much more expensive and would require larger storage and transportation facilities. After careful analyses of the potential risk and benefits, WHO maintains the acceptance of thimerosal in vaccines for the global market, pending further data.

4.2. Rotavirus vaccine

Rotavirus infection is a diarrhoeal disease that kills up to 800,000 children world-wide each year [22,23]. Although rotavirus accounts for 55,000 children admissions to hospital per year in the US, it accounts for only 20–40 of the global deaths [23]. In contrast, 1 in 200 infected children die from dehydration or related complications in developing countries [22,23].

Two years ago, the US manufacturer of a vaccine to prevent rotavirus infection, withdrew the product from the market and this led to the withdrawal from the market by the manufacturers and then withdrawal of recommendation for use by regulatory bodies. This withdrawal was prompted by reports of 'temporal association' of vaccination with increased risk of intussusception,. However, two new studies suggest that the risk–benefit calculations in 1999 may have been in error, thus calling to question the basis for the original reports [24,25]. While this decision may have been appropriate for the circumstances in the US, it would be very much less so for other settings. An argument can be made for the evaluation of the same vaccine in a population with a higher disease burden and case specific mortality than is currently reported in the US. While there may be a political risk with such a decision, an informed local ethics committee should be able to evaluate the potential cost-benefit of such an intervention. There has always been a sense that a vaccine has to be approved for use in the US or at least in the country of production otherwise it cannot be used elsewhere. Yet the risk–benefit calculations are strikingly different for rich and poor countries. There is a need to promote the capacity to perform risk–benefit analysis in less developed countries and making this an integral part of vaccine utilisation services.

5. Improving cost effectiveness of vaccines

Although cost is not the only barrier to adopting the use of new vaccines in poor countries, a significant reduction in unit cost or delivery cost may make some of these products more readily within reach of several countries (Tables 3 and 4).

5.1. Local production of vaccines in developing countries with supervision and support from developed countries

Pharmaceutical firms that are based in developed countries manufacture a large proportion of the vaccines in current use, globally. The unit cost of these vaccines make it unaffordable to several developing countries and despite the substantial subsidisation that is achieved by bulk purchase of these vaccines by international agencies such as UNICEF for developing countries, the price is still not within the reach of several countries.

The multinational pharmaceutical firms should be encouraged to establish branches in regions of developing countries, where labour is relatively cheap and this could reduce substantially, the unit price of these vaccines. While it will not be feasible to set this up at country level, regional zoning can be established in areas with similar disease

Table 3
Tools for advocating use of vaccines

Generation of local burden of disease data (morbidity and mortality)
Demonstration of immunogenicity and/or efficacy and safety of vaccine in the population
Presentation of information to opinion leaders and policy makers
Public enlightenment through appropriate media (television, radio, newspapers, public meetings)

Table 4
Improving cost effectiveness of vaccines

Encouraging local production in developing countries with supervision from established firms in developed countries
Economics of vaccine dosage
Evaluation of fewer doses
Evaluation of fractional dose regimen
Alternative, cheaper regimens, e.g. immunisation during pregnancy, neonatal immunisation
Improving the use and availability of combination vaccines

epidemiology with active involvement of the governments, so that joint ownership is established from the onset. This approach should not in any way compromise the quality of the vaccines as the firms will be expected to set up and provide expert supervision at these production sites. This approach would foster the development of 'trust' in immunisation programs and also has the added advantage that at least some developing countries will begin to have a sense of ownership of these products and will not continue to perceive the concept of vaccination as entirely foreign.

5.2. Economics of vaccine dosage

Protein conjugate vaccines that have recently been licensed such as the *H. influenzae* type b and 7-valent pneumococcal vaccines have been recommended for use in a four-dose schedule in infants in the US. It remains contentious however, if so many doses of these vaccines are required to achieve protection [26,27]. Although the Hib vaccine is being used in the UK in a three-dose schedule, there has been no firm evidence to suggest that this approach is less optimal. Furthermore, there have been studies elsewhere suggesting that immunogenicity can be achieved using a fractional dose of the Hib vaccine, although efficacy of this approach has not been evaluated [27]. In addition, these conjugate vaccines have an enormous potential in their value as they impact carriage, and consequently provide herd immunity, even when coverage is very low. In a recent report from the Veneto region of Italy, a coverage of 26% for *H. influenzae* type b primary vaccination in 1-year-old children plus a 31–53% catch-sup coverage in children 1–4 years of age resulted in a 91% reduction of Hib invasive disease in children <5 years of age [28].

More recently, low dose plasma derived hepatitis B vaccine, which is affordable to most developing countries, was very successful in controlling endemic hepatitis B infection, where the virus is predominantly spread by horizontal transmission among infants and young children [29,30].

5.3. Fractional dosage

Although the Hib and pneumococcal conjugate vaccines have been licensed, the minimal quantity of antigen required to induce immune protection continues to be a subject of intense research. Some studies have demonstrated the immunogenicity of fractional doses as low as 1/10th of the recommended dose. In a recently reported study from South Africa, a 10-fold dilution of the Hib conjugate vaccine elicited antibody response that was higher than the recommended dose and had equivalent antibody avidity.

In theory, this observations would suggest that such fractional dose regimens would perform as well as the recommended dose in offering protection from invasive disease. However, adopting the use of fractional dose will constitute 'off label use' of these products, with potential liability consequences. However, from a scientific standpoint, it is an intriguing observation that may warrant further evaluation for economic reasons.

5.4. Combination vaccines

Another approach to reducing the cost of vaccine delivery is the use of combination vaccines. Although there may be an increase in the production cost, these should be offset by the gains made from the decrease in storage space, transportation and savings from the use of less clinical consumables such as needles and syringes. In addition, coverage may be increased, particularly in communities where the current immunisation programs are seen as offering too many injections. On-going research activities in formulating combination vaccines have focused mainly on vaccines in routine use in developed countries, such as the pentavalent (hepatitis B, diphtheria, tetanus, acellular pertussis and Hib vaccine combination).

The use of combination vaccines in developing countries has not been explored. Some combination vaccines in routine use in developed countries have a potential for reducing enormous disease burdens in developing countries. Congenital rubella syndrome is an under-recognised public health problem in many developing countries. In 1996, the mean of the estimates of the total number of congenital rubella syndrome in developing countries was approximately 110,000. As of 1997, less than one-third of developing countries included rubella vaccine in their national immunisation programme. In settings where there is already high coverage of measles vaccine, an ideal opportunity exists for the inclusion of rubella and mumps [32].

In developing vaccine combinations, regional disease epidemiology, immunisation practices and cost must be key determinants. The potential of combination vaccines is yet to be explored to the benefit of developing countries. A combination of measles, mumps, rubella and yellow fever vaccines could conceivably be beneficial and cost saving in most settings.

6. Conclusions

Developing countries often bear the greater brunt of infectious diseases but due to minimal economies, poor manufacturing capabilities, they have to depend on developed countries for the production and procurement of vaccines [30]. There is a need to strengthen collaborative efforts between developing and developed countries in the basic science research, evaluation and implementation of vaccine use.

Disease surveillance systems must be developed to inform and assist governments and policy makers in developing countries to determine intervention needs and formulate appropriate policies. Aetiology specific surveillance systems may be laborious and expensive to set up but they are most informative. Since cost may prohibit the establishing surveillance sites in all countries, regional sentinel sites can be

developed based on disease epidemiology, with the participation and support of all countries in the region. Information from such sites could then be used for defining needs and formulating policies. A cheaper but less sensitive approach, which could be used at district levels, is the disease syndrome definition. This system classifies illness by a group of signs and symptoms to suggest possible aetiology. While this approach is useful for the clinical definition of conditions like acute poliomyelitis, which is described by a triad of unilateral flaccid paralysis with atrophy, unimpaired sensation and a history of acute onset [33], syndromic definitions for other conditions such as acute lower respiratory infections are much less aetiology-specific. Creation of sentinel sites in different country regions that could provide this service with support and supervision from developed countries or international agencies should be strongly encouraged. The development of this service will be invaluable for monitoring the effectiveness of current immunisation services and evaluation of the need for the introduction of newer vaccines.

As vaccines are frequently under-utilised, a recent hypothetical model highlights the parameters that might be modified to increase the probability of uptake. The most sensitive factors include vaccine cost, per-capita GDP, infrastructure, perception of disease burden and population size [31,34]. A few of these might be influenced to increase the probability of vaccine uptake. Although closely interrelated, improving vaccine uptake through these factors will require a multifaceted input from several sources. For example, although it may be easy for donors to subsidise the unit cost of vaccines, improving per-capita GDP would be a lot more challenging, but in the long run, critical to the sustenance of quality health services and immunisation, in particular.

The ultimate goal remains the discovery and development of single dose combination vaccines that can be administered either by oral or mucosal routes and do not require a cold chain, making the task of immunising infants very simple, convenient and more economical. However, we must grapple with the multiple hurdles that we currently face. There is a need for concerted global effort to develop systems for the provision of vaccines to the world's children that are neither profit-driven nor politically motivated but geared towards the improvement of survival and the quality of childhood.

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EXHIBIT E

"Nasal mucociliary clearance as a factor in nasal drug delivery."
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Nasal mucociliary clearance as a factor in nasal drug delivery

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Abstract

The nasal mucociliary clearance system transports the mucus layer that covers the nasal epithelium towards the nasopharynx by ciliary beating. Its function is to protect the respiratory system from damage by inhaled substances. Impairment of nasal mucociliary clearance can result in diseases of the upper airways. Therefore, it is important to study the effects of drugs and drug excipients on nasal mucociliary clearance. A large number of methods are used to assess mucociliary clearance. These methods study the effects of drug and excipients on the mucociliary system in vitro or in vivo in animals and humans. In some cases, the results of different in vitro and in vivo measurements do not correlate well. In vitro methods, especially ciliary beat frequency measurements, have been demonstrated to be valuable tools for toxicity screening. However, in vivo studies are essential to confirm the safety of nasal drug formulations. Nasal mucociliary clearance also has implications for nasal drug absorption. Drugs are cleared rapidly from the nasal cavity after intranasal administration, resulting in fast systemic drug absorption. Several approaches are discussed to increase the residence time of drug formulations in the nasal cavity, resulting in improved nasal drug absorption. However, more experimental evidence is needed to support the conclusion that this improved absorption is caused by a longer residence time of the nasal drug formulation. © 1998 Elsevier Science B.V.

Keywords: Nasal mucociliary clearance; Nasal drug delivery; Ciliary beat frequency; Absorption enhancers; Cyclodextrins; Preservatives

Contents

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1. Introduction

Nasal delivery is a promising alternative for systemic administration of drugs that are poorly absorbed via the oral route. The nasal epithelium has a relatively high permeability [1–3], and only two cell layers separate the nasal lumen from the dense blood vessel network in the lamina propria. These factors make nasal drug administration an attractive delivery route, but they also make the nasal mucosa vulnerable to adverse effects of drugs and excipients in nasal drug formulations. The nasal mucociliary clearance system is particularly susceptible to damage. This can have serious consequences, because the mucociliary clearance plays an important role in the protection of the respiratory system. Noxious substances entrapped in the mucus layer of the nasal cavity are transported towards the nasal cavity by ciliary movement. Nasal mucociliary clearance also largely determines the absorption profile of nasal drug delivery, since the residence time of drugs administered to the nasal cavity is limited by mucociliary clearance [4]. Studying the nasal mucociliary clearance system is therefore important in order to optimize nasal drug delivery, with respect to both safety and the amount and rate of absorption.

2. Nasal mucociliary clearance

2.1. Nasal mucosa

The human nasal cavity is lined with three types of epithelia; squamous, respiratory and olfactory. The mucosa in the anterior part of the nose is squamous and without cilia. Within 1 cm of the nostrils, a transitional epithelium is found that precedes the respiratory epithelium. The olfactory epithelium is found in the posterior part of the nasal cavity. The respiratory epithelium is the major lining of the human nasal cavity and is essential in the clearance of nasal mucosa by the mucociliary system. This epithelium is composed of ciliated and non-ciliated columnar cells, goblet cells and basal cells [5]. The columnar and goblet cells are found on the apical side of the cell layer, next to the lumen of the nasal cavity. Basal cells are found adjacent to the basal lamina, on the basolateral side of the epithelium. The lamina propria is located beneath the basal lamina and contains many blood vessels, nerves and glands.

2.2. Cilia

Cilia are motile hair-like appendages extending from the surface of epithelial cells (Fig. 1). They contain an axoneme (Fig. 2), i.e. a bundle of microtubules arranged as nine outer doublets and a central pair (nine + two arrangement). The axoneme is surrounded by a specialized extension of the cell membrane, the ciliary membrane [6]. It is assembled in a fixed pattern at the cell surface, above the basal bodies [7]. A doublet microtubule is composed of two subfibres, A and B. Subfibre A has inner and outer dynein arms with ATPase activity. Adjacent doublets are connected by nexin lines, and radial spokes connect the microtubule with the two single microtubules in the center. Movement of the cilium is generated by sliding movements of the microtubules [8]. The dynein arms on subfibre A of one

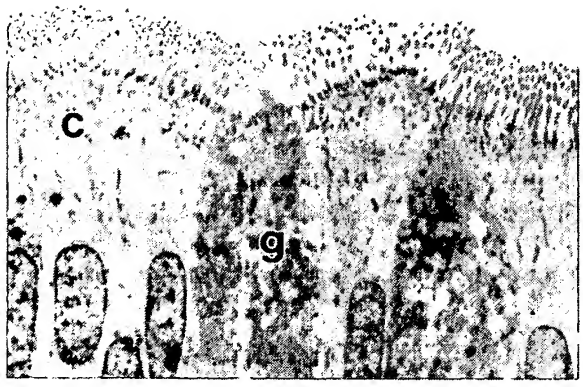

Fig. 1. Transmission electron micrograph of respiratory nasal epithelium of the rat showing ciliated (c) and goblet (g) cells (2500× enlargement).

doublet walk along subfibre B of an adjacent doublet as ATP is hydrolysed. Active sliding of the outer doublets relative to one another generates curvature, and the force between adjacent doublets is generated by dynein cross-bridges. The radial spokes resist this sliding motion, which, instead, is converted into local bending. Nexin, a highly extensible protein, keeps adjacent doublets together during the sliding process [9,10].

Cilia range in length between 5 and 10 μm and in width from 0.1 to 0.3 μm (Fig. 1). The number of cilia per cell is approximately 200, with a density of six–eight cilia per μm^2 [11]. The beating of a cilium has three phases, an effective stroke, during which the cilium is extended maximally, the rest phase, in which it is parallel to the cell surface, and the recovery stroke. Ciliary beat frequency is under cellular control and is, on average, 15–20 Hz in the respiratory tract of mammals [12]. The average beat frequency of human nasal cilia is 10 Hz, as measured in an in vitro test system [13]. Cilia beat in close coordination in a metachronal wave by adjusting their frequency and phase of beating in response to neighbouring cilia [14].

The ciliary beat frequency is regulated by several factors: Temperature, intracellular Ca^{2+} and cAMP levels, and by extracellular ATP. The ciliary beat frequency of human nasal cells in vitro increases with increasing temperature, between 5 and 20°C. Between 20 and 45°C, it was found to stabilize at around 8–11 Hz, or around 14 Hz between 32 and 37°C [13,15]. The temperature dependency of cilia is mostly regulated by axonemal enzymatic components, while the ciliary membrane has little regulatory effect [13].

Intracellular Ca^{2+} levels also modulate ciliary beating. Elevated levels of intracellular Ca^{2+} increase ciliary beat frequency, while Ca^{2+} depletion

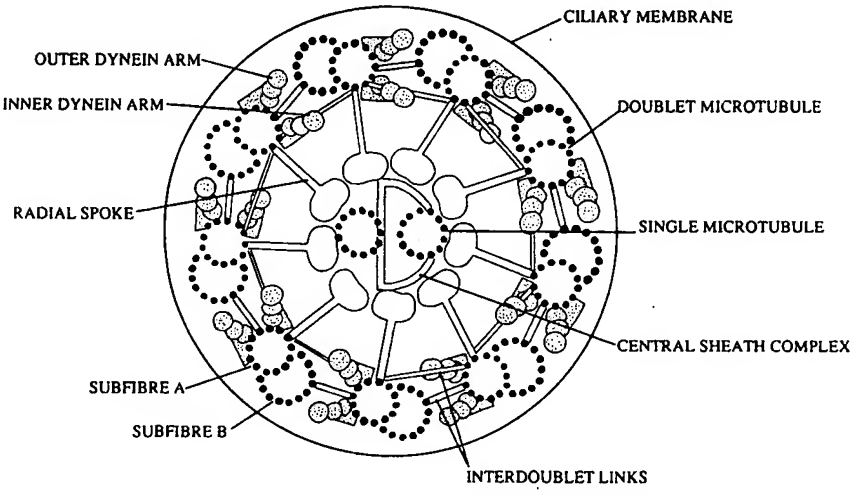

Fig. 2. Cross-section of a cilium. Each outer doublet microtubule consists of two subfibres (subfibres A and B). The A subfibre bears the inner and outer dynein arms and the radial spokes. The spoke heads are oriented towards the two single microtubules of the central sheath complex. Two sets of links join adjacent doublets. Reprinted from ref. [11] with kind permission of Elsevier, Amsterdam.

decreases ciliary activity [16]. In single cell systems, changes in intracellular Ca^{2+} were found to be kinetically coupled to changes in ciliary beat frequency, indicating that Ca^{2+} is an essential messenger in the regulation of ciliary beat frequency [17]. Stimulation of a single cell leads to a wave of increased beat frequency across a cell culture and the increase in ciliary beat frequency is preceded by an increase in intracellular Ca^{2+} [18,19].

Extracellular ATP can increase the intracellular Ca^{2+} level in cell cultures, resulting in an increased ciliary beat frequency [20]. Different Ca^{2+}-dependent mechanisms are believed to regulate ciliary activity: The action of ATP, via a purinergic receptor coupled to transmembrane influx of Ca^{2+} and the action of prostaglandin, via receptor-mediated release of intracellular sequestered calcium [20]. Mechanostimulation by foreign particles or mucus on the cilia can increase the ciliary beat frequency [21]. Dual regulation of ciliary beat frequency can be beneficial for mucus transport, because mechanical stimulation would provide local control to elevate beat frequency in the immediate vicinity of the mucus load, whereas neurohormonal stimulation would regulate the general level of ciliary activity throughout the airways [22]. The ciliary beat frequency is also increased by increasing the levels of intracellular cAMP and cGMP [23–25]. Cyclic AMP and Ca^{2+} may regulate ciliary beat frequency by acting at a common site within the cell, possibly regulating the rate at which the axoneme can use ATP, or the availability of ATP to the axoneme [23].

Bradykinin, histamine, ATP and UTP all elevate intracellular Ca^{2+} in human airway epithelia through receptor-mediated activation of phospholipase C, followed by inositol triphosphate production [25]. The intracellular messenger, inositol triphosphate, acts as both an intracellular and intercellular messenger in respiratory cells, by moving through gap junctions to coordinate a coupled increase in ciliary beat frequency [22]. Many substances, such as ethanol, stimulate ciliary activity by releasing nitric oxide via up-regulation of nitric oxide synthetase [26]. Nitric oxide synthetase activity was found to be localized in ciliated cells of the human nasal epithelium [27]. A lack of nitric oxide was observed in the upper airways of patients with chronic sinusitis, who had functional and morphological changes of the mucociliary clearance system [28].

2.3. Mucus

The respiratory epithelium is covered by a mucus layer. This layer is divided into two distinctive layers, the periciliary layer and a more gellous upper layer. The periciliary layer is a low viscosity fluid with a thickness that is slightly less than the length of an extended cilium. It is probably formed by epithelial cell exudate [29]. The periciliary layer is covered by a more viscous upper layer of about 0.5–5 μm deep [30], which is secreted by the goblet cells [31]. It contains mucins, which are glycoproteins that vary in molecular weight from a few hundred Daltons to more than $1 \cdot 10^7$ Daltons [32]. Approximately 80% of the weight of the mucin molecule consists of carbohydrates [33]. About 3% of the mucus layer consists of mucins, while 90–95% consists of water, with electrolytes, serum proteins, immunoglobulins and lipids [31,34].

The mucus-secreting goblet cells are part of the nasal respiratory epithelium and are found in between fields of ciliated cells. Mucus is expelled from the goblet cells as highly condensed granules by exocytosis [31]. Upon exocytosis, the secreted granules undergo massive swelling and the mucins are mixed and annealed to form a viscoelastic gel that is transportable by the cilia [31]. When the mucus glycoproteins are dissolved in water, an entangled network is formed. This mucus gel is stabilized by non-covalent interactions between mucin molecules, and collapses when the disulphide bonds are reduced [31].

Mucus exhibits non-Newtonian behaviour, i.e., it possesses both viscous (fluid) and elastic (solid) properties and it is, therefore, described as viscoelastic [34]. The viscous properties enable mucus to efficiently accept the energy transfer from the cilia, while its elastic properties enable it to relax sufficiently to be propelled. For efficient transport, the elasticity is the most important parameter, with an optimal range of elastic moduli of 1–2 N/m^2 [35,36]. The extent of the entanglement of the mucin polymers is regulated by the degree of hydration of the gel. This is dependent on its macromolecular composition and ionic content [34]; by modulating

these factors, the rheology of the mucus layer can be changed.

2.4. Mucociliary clearance

The function of the mucociliary clearance system is to remove foreign substances and particles from the nasal cavity, thus preventing them from reaching the lower airways. The mucociliary clearance system has been described as a "conveyer belt" in which ciliated cells provide the driving force, and mucus performs as a sticky fluidic belt that collects and disposes of foreign particles [31]. The efficiency of the mucociliary clearance system is therefore dependent on the physiological control of the ciliated cells and on the rheological properties of the mucus blanket.

The mucus layer is propelled by the cilia towards the nasopharynx. A single cilium swings upwards during its effective stroke and the tip penetrates the mucus. The cilium will transmit its energy to the slower-moving mucus layer, and the tip then continues downwards into the periciliary fluid. As the movement of a single cilium is slowed by the inertia of the slower-moving mucus blanket, other cilia catch up with it and transfer their energy to the mucus as well [14]. While the effective stroke propels the overlying mucus forward, the underlying periciliary fluid only moves forward and backwards during the beat cycle. Metachronal coordination of the cilia maintains a continuous forward thrust on the mucus. The presence of several metachronal waves under a blanket of mucus spreads the propulsive movement so that the whole blanket moves as a unit. The mucus blanket in the nose is transported towards the nasopharynx, where it is swallowed [7].

Normal mucociliary transit time in humans has been reported to be 12 to 15 min [37]. Transit times of more than 30 min are considered to be abnormal, and are indicative of impaired mucociliary clearance [37–39]. The average rate of nasal clearance is about 8 mm/min, ranging from less than 1 to more than 20 mm/min [37]. In a study of 46 healthy subjects, mucociliary transport was found to be independent of sex or age [40]. Radiolabelled nasal sprays exhibit biphasic clearance from their site of deposition [11]. The first phase of clearance lasts about 15–20 min,

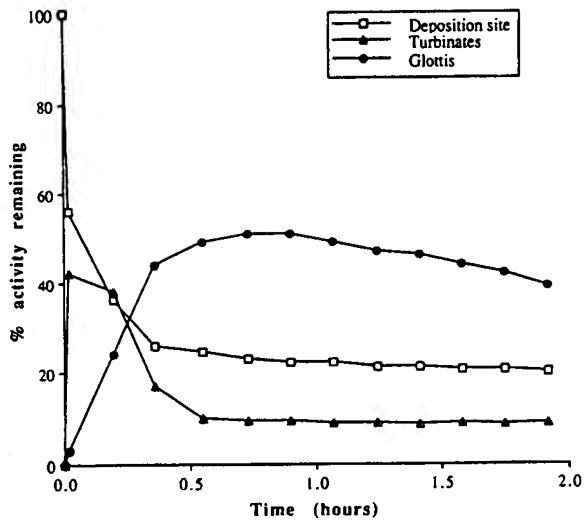

Fig. 3. Deposition and clearance of ^{99}Tc-labelled diethylenetriamine pentaacetic acid solution administered to the nasal cavity of human volunteers. Reprinted from ref. [139], with kind permission.

in which about 50% of the administered dose is cleared from the ciliated respiratory mucosa (Fig. 3). The second phase is slower, and removes the part of the nasal spray that is deposited on the non-ciliated vestibule and anterior septal area.

2.5. Pathophysiology of mucociliary clearance

The function of mucociliary clearance is to protect the nose and the lower airways from damage by inhaled noxious substances, therefore, the impairment of this system is potentially harmful [4]. Impaired mucociliary clearance causes longer contact times of the airway mucosa with bacteria and viruses, which could lead to infections of the respiratory tract. The airways can also be exposed for longer to irritating or carcinogenic substances, resulting in damage to the mucosa. The efficiency of the nasal mucociliary clearance system depends on three factors: (a) The amount of ciliary input, determined by the length and density of the cilia and their beating frequency; (b) the amount of mucus and the depth of the periciliary layer and (c) the viscoelastic properties of the mucus [14]. Changes in

any of these three parameters might cause impairment of the mucociliary clearance system.

Several pathological conditions exist in which mucociliary clearance does not function properly [8]. In patients with primary ciliary dyskinesia, who have no or dyskinetic beating cilia, infections of the respiratory system frequently occur [8]. Patients with cystic fibrosis also have an impaired mucociliary clearance system, compared with healthy persons [41], but their cilia are normal and function well [8]. The mucus of cystic fibrosis patients has a reduced water content, and the transport rate of this mucus was observed to be delayed in vitro [42]. In the case of viral and bacterial infections, the mucociliary clearance system is compromised, most likely due to a loss of cilia, but possibly also to a change in the rheologic properties of the mucus [43]. In patients with the common cold, loss of cilia was found in the first week, but the cilia recovered within three weeks [44]. Hospitalized patients in intensive care units often have impaired mucociliary transport, which is associated with the development of pneumonia and retention of secretion [45]. In diabetes mellitus patients, who are more susceptible to nasal infection diseases, the nasal mucociliary clearance time was found to be significantly larger than in a group of non-diabetic controls [46].

From these studies of pathological conditions, it is obvious that recurrent respiratory diseases are related to impaired mucociliary clearance, but it is not clear whether impaired mucociliary clearance is the cause or the result of respiratory diseases [47]. It seems that the existence of functioning cilia is important for the functioning of the respiratory defence system, but not of vital importance [8]. Evidence for this is provided by the finding that the life expectancy of patients with primary ciliary dyskinesia seems to be normal, provided that respiratory infections are treated adequately [48,49]. Coughing functions as a substitute defence mechanism of the lung when the mucociliary clearance system is defective [8]. Furthermore, damaged nasal epithelium is able to recover completely. In rats and rabbits, complete regeneration of the nasal mucosa was observed within five to ten days, provided that the basal cells and basement membrane were left intact [50,51]. When the entire epithelium was removed, full regeneration of the nasal mucosa was observed within six weeks in rabbits [50].

3. Assessment of mucociliary clearance

A large number of methods to assess mucociliary function have been described (Table 1) [4,11]. These methods study the mucociliary system as a whole (in vivo), or as its separate components (in vitro or in vivo), i.e., the ciliary activity and the mucus layer.

3.1. Methods to measure ciliary beat frequency in vitro

Several methods have been described to measure the beat frequency of moving cilia from the upper airways in vitro [12,52]. Some of these methods are based on cinematographic or video detection. In this method, a video was recorded and afterwards evaluated at low speed, to determine the ciliary beat frequency [53–55]. Video recordings of beating cilia have also been digitized, and the resulting images were used for computer analysis [17,26]. By placing a pinhole photodiode on a video recording of beating cilia, a photoelectric signal is obtained, which can be further analysed by computer [53,56–60]. With a high speed digital video camera, analysing the ciliary beat frequency and metachrony of cultured cells or epithelial sheets is possible, with high temporal and spatial resolution [61–63]. The video camera can be connected to a fluorescence imaging system, making it possible to study the physiological control of ciliary activity by simultaneously measuring ciliary beat frequency and intracellular ions or messengers [64].

Ciliary activity has also been assessed by photoelectronic detection, in which either reflected or transmitted light is used. Measuring variations in the light reflected is possible both in vivo and in vitro [65,66]. The reflections can originate from the cilia and/or from the mucus layer, and these may contribute to the reflected image. Back-scattered laser light that was reflected has been used to detect the frequency of ciliary beating, by calculating the Doppler shift of back-scattered photons from the cilia [67,68]. A new laser light scattering system to measure ciliary beat frequency has been developed to determine the metachronal wave period and direction [69]. This method is based on a heterodyne mode correlation analysis light scattering system, with two spatially separated focal spots. The back-scattered signals from two adjacent groups of cilia are cross-

Table 1
Methods to determine the ciliary beat frequency

Method	Detection	In vivo/In vitro	Species and tissue	Reference
Video	Slow speed monitoring	In vitro	Human nasal	[53–55]
	Digital computer analysis	In vitro	Human nasal	[59,62,63,112]
			Bovine bronchial	[26]
			Sheep tracheal	[17]
	Pinhole photoelectric	In vitro	Human nasal	[53,56–58,60,74]
Photoelectric	Reflected light	In vitro	Human nasal	[81]
			Rat tracheal	[81,98]
			Guinea pig tracheal	[81]
		In vivo	Human nasal	[116,117]
			Rabbit nasal	[66,105,114,115,117,165,251]
			Guinea pig tracheal	[98,170]
Photoelectric	Back-scattered laser light	In vitro	Dog tracheal	[69]
			Sheep tracheal	[69]
		In vivo	Dog tracheal	[68,163]
Photoelectric	Transmitted light	In vitro	Human nasal	[71,72,75,83,180]
			Human sphenoidal	[88]
			Human adenoidal	[73,80,85,86]
			Chicken tracheal	[52,80,85,99–102], [130,186,252]
			Rat tracheal	[89,91–97,170,225]
			Rabbit tracheal	[16,103,104,107,108,253]
			Guinea pig tracheal	[16,106–108,165,253]

correlated to give the phase distribution of the ciliary activity [69]

A widely applied photoelectronic detection method to measure ciliary activity is the transmitted light technique [70]. This method is used in a large number of studies (Table 1) [52,71–75]. In principle, light is transmitted through ciliated epithelium, and the changes in light intensity due to ciliary movements are detected by a photosensitive cell. The transmitted light technique allows a precise and direct measurement of the ciliary beat frequency, and an average value of a group of beating cilia is obtained. The number of cilia measured depends on the system and the ciliated samples, and varies between 1 and 2000 [52,76–79].

Photoelectronic detection is probably the most convenient means of quantifying ciliary beating. The ciliary beat frequency gives a complex spectrum of frequencies, but fast Fourier transform analysis of the analog signal gives a power spectrum of the fluctuating frequency [77]. A disadvantage of using transmitted light is that only the ciliary beat frequency of cilia at the edges of a piece of tissue explant can be measured. However, in cell cultures, it is possible to transmit light through the cell monolayer.

Ciliary beat frequency is frequently measured at body temperature (37°C), while the physiological range of nasal mucosa temperatures lies between 31 and 35°C. However, between 32 and 40°C, the nasal ciliary beat frequency was found to be independent of temperature [15,52]. Optimal ciliary beat frequency was observed between pH values of seven and ten. Values lower than pH 6 and higher or equal to pH 11 resulted in large decreases in the beat frequency of chicken embryo trachea. In isotonic solutions, the ciliary beat is best preserved [52].

3.1.1. Human tissue

Human ciliated tissue explants have been used in a large number of studies to determine their ciliary beat frequency. Human tissue is preferable, because species differences in ciliary activity have been reported [80–82]. Human nasal ciliated tissue can be obtained by brushing the inferior nasal turbinates or the anterior nasal wall without anaesthesia

[13,15,56,58–60,71,75,83,84], or under local propofol anaesthesia [74]. Propofol does not affect ciliary activity in human nasal ciliated epithelium [58]. Ciliated tissue can also be obtained from biopsies or from patients undergoing surgery [53,57,62], but local and general anaesthetics are known to decrease ciliary beat frequency [74,84]. Moreover, the nasal mucosa of these patients is frequently in a pathological condition. Therefore, the effects of drugs on ciliary beat frequency of human adenoid tissue are also studied [73,80,85,86].

A disadvantage of surgically obtained ciliated tissue is that it can only be conserved for a limited time. Therefore, a method has been developed to cryopreserve ciliated tissue [87,88]. As an alternative to cryopreservation, a perfusion chamber has been designed in which it is possible to keep nasal ciliary beat frequency at a constant level for 4 h in a controlled and stable environment [58].

3.1.2. Animal tissue

In vitro ciliary beat frequency studies in animal tissues are often performed with tracheal explants, using photoelectric detection of transmitted light. The species most frequently investigated are rats [81,89–98] and chicken embryos [52,80,85,99–102]. Ciliated tissue can also be obtained from rabbits and guinea pigs [103–106]. The advantage of chicken embryos is that they are cheap and easy to obtain. It is possible to perform ten–fifteen different measurements on the tissue of one animal.

Species differences exist with respect to the effects of substances on ciliary beat frequency. The effects of chinoin-170, an antitussive compound, on the ciliary beat frequency of rat trachea in vitro were inhibitory, but the ciliary beat frequency of human mucosal explants was not changed by chinoin-170 [98]. The same difference in effects was found for preservatives on rat trachea explants and human nasal mucosa [81]. Nevertheless, in a number of studies, the effects of drugs and excipients on the ciliary beat frequency in vitro have been found to be similar for chicken embryo tracheas, human adenoid tissue and human sphenoidal sinus mucosa tissue [80,85,88].

3.1.3. Cell cultures

Cell cultures of a variety of animal respiratory epithelia have been used to study the ciliary beat frequency [21]. The effects of substances on ciliary beat frequency were investigated with the transmission photoelectric technique on cell cultures of rabbit trachea [107,108]. The rabbit tissue explants were plated on coverslips so that the light could pass through the tissue culture.

In human airway epithelial cells, generating fully differentiated ciliated cells in cell culture is more difficult. A culture system of outgrowths of human nasal polyps has been developed that contains 50% ciliated cells, of which 85% had a ciliary beat frequency of 13.3 Hz [109]. In a modification of this system, cells were cultured on a floating collagen gel, and a pseudostratified respiratory-type epithelium, similar to that in an in vivo situation, was developed [110]. The mean ciliary beat frequency of the culture was 12.2 Hz, and could be maintained for more than 35 days. The ciliary beating of a cell culture on cover glasses was studied using high speed video microscopy [63]. Although the ciliated cells plated on cover glasses had a ciliary beat frequency of 20.6 Hz, their beating was not coordinated. The ciliary beat frequency of monolayers plated on glass coverslips decreased linearly from the first day after plating, and it was best preserved in cultures with a high seeding density [111]. Using an inverted microscope and a high speed video system, it was possible to study the ciliary beat frequency of human respiratory cells cultured on floating collagen gels [112]. Intercellular coordination of ciliary beat frequency was still absent on day 14 of the cell culture. Intracellular coordination of ciliary beating was increased with elongation of the cilia, but it was observed that high ciliary beat frequency values of 19.6 Hz were not indicative of mature cilia [112]. The high ciliary beat frequency value may be partly due to cilia that are too short to reach the mucus layer. An air interface culture has been developed to culture human respiratory cells. In this modified cell culture set-up, mature cilia and mucus production were observed after three weeks in a culture of bronchial cells [113], or after twenty days in a nasal cell culture system [63].

The developments in culturing human nasal epithelial cells are very encouraging, and show that measuring physiologically relevant ciliary beat frequency values in cell cultures is possible. However, to our knowledge, there are no studies yet available in which the effects of drugs or drug excipients on these systems have been investigated.

3.2. Methods to measure ciliary beat frequency in vivo

The ciliary beat frequency can be measured photoelectrically in rabbits in vivo [66]. The rabbit is anaesthetized, and an opening is made in the skull to expose the mucosa of the maxillary sinus. Light reflected by the mucociliary activity is observed through a window with a microscope, and the signal is detected photoelectrically. This method has been used to study the effects of intravenous administration of xanthine derivatives and cAMP, and to investigate if inhalation anaesthetics affect the ciliary beat frequency in vivo [114,115]. Disadvantages of this method are the use of general anaesthesia throughout the experiment, and the possibility of trauma to the mucosa as a result of the surgery.

These disadvantages can be overcome by measuring the effects on mucociliary activity in man in vivo without surgery or anaesthesia [116]. The mucociliary activity is measured via light reflection by the mucosa. Light is transmitted to the mucosa by an optical fibre in a tube inserted into the nostrils, and the reflected light from the mucosa is transmitted through the same tube. To avoid interference by movements of the head, the mucosa is immobilized by fixing the head of the volunteer in a head support. The method showed good reproducibility, but the mucociliary wave frequency in vivo did not correlate well with the corresponding ciliary beat frequency in vitro [116]. The analysis of reflected light by the mucociliary activity in vivo can be improved by fast Fourier transformation of the photoelectric signal, which results in greater sensitivity and better detection of the contribution of artifacts to the signal [117].

3.3. Methods to measure mucociliary transport in vitro

An in vitro method to study mucociliary transport is the excised frog palate model (Table 2). The ciliated epithelium of the frog palate is a frequently used model to evaluate the role of rheological properties of mucus on mucociliary transport velocity [36,118–120]. The bullfrog (*Rana catesbeiana*) has a broad, flat area of mucosa on its palate, which can be used to test the mucociliary transport rate in vitro. The ciliated mucosa of the palate closely resemble that of mammalian respiratory epithelium with respect to morphology, function and histochemistry [119,121]. However, in the absence of mucus or extracellular Ca^{2+}, the cilia of the frog palate do not beat, in contrast to cilia of the mammalian respiratory epithelium [122,123].

There are two kinds of frog palate experiments; mucus-depleted and mucus non-depleted. The results of these two methods are not always comparable [118]. The depleted frog palate model has been used to determine the role of rheological properties in mucociliary transport. In these studies, the palate is depleted of mucus, followed by the application of exogenous mucus samples to the palate to measure their transport rates [42,124,125]. Not only mucus samples, but also viscoelastic gels have been tested with this method, for example, gels used in nasal drug formulations [118,126–129].

In the non-depleted frog palate model, the mucus

Table 2
Methods to determine mucociliary transport in vitro and in vivo

Species and tissue	In vivo/in vitro	Tracer	Reference
Frog palate (mucus-depleted)	In vitro	None	[120,125,166]
		Aluminium disk	[42,193,254]
		Charcoal powder	[127,128]
		Graphite particles	[129]
Frog palate (mucus non-depleted)	In vitro	Graphite particles	[99,130–133]
Human nasal	In vitro	Graphite particles	[134]
Rat nasal	In situ	Charcoal powder	[125,166]
	In vivo	Fluorescent latex spheres	[160,161]
Rabbit nasal	In vivo	Dye	[240]
Chicken nasal	In vivo	Dye	[169]
Rat tracheal	In vivo	Carbon particles	[162]
Guinea pig tracheal	In vivo	^{99}Tc erythrocytes	[183]
Dog tracheal	In vivo	Iron particles	[164]

layer is still present. This layer is essential for transport, and it also offers an intact barrier that protects the underlying cilia. Because of these characteristics, the non-depleted frog palate model is used to study the effects of nasal drug formulations and their components on an intact mucociliary clearance system [68,99,130–133].

A novel in vitro method was developed, in which surgically removed human nasal turbinate tissue was used to determine nasal mucociliary transport [134]. The transport of graphite particles was detected by a video camera and processed by computer image analysis, employing a software program to track the movements of individual graphite particles. The mucociliary transport in the frog palate model has also been determined with this method [133].

Mucociliary transport is a function of the rheological properties rather than of the chemical properties of the viscoelastic mucus [35,135]. A mathematical description of this function has been developed and tested by Yu et al. [126]. Methods used to evaluate the rheological or flow properties of mucus have been reviewed by Wanner [12] and Marriott [136].

3.4. Methods to measure mucociliary transport and clearance in vivo

3.4.1. Human studies

A summary of the methods used to determine nasal mucociliary clearance in humans is given in Table 3. Different features of the mucociliary clearance system have been studied, and a general differentiation into two categories can be made. The first one is the determination of the total nasal clearance of a deposited dose, by measuring the clearance of a radiolabelled solution from the nasal cavity. In this method, a radiolabelled solution is deposited in the nose via nose drops or a nose spray [137]. The clearance of radioactivity from the nasal cavity is measured with a gamma camera. This method is commonly used to determine the residence time of drug preparations in the nose (Fig. 3) [138–142]. The second category involves methods to determine the mucus flow rate or mucociliary transport time, by measuring the transport time or speed of markers placed on the nasal mucosa (Table 3). Radiolabelled particles are administered to the ciliary epithelium of the nose, and the transport of the particle is followed by a gamma camera to calculate the speed. This method has been modified by decreasing the size of the particle to prevent impairment of ciliary activity and by the use of lower activities [143–145]. Despite these efforts, the main drawback of this method is the administration of radioactive material to volunteers. As an alternative, radiopacque Teflon disks were administered nasally and detected by roentgenography [146,147].

One of the most simple and inexpensive methods of estimating mucus flow in vivo is by using dyes as markers. Drops or particles coloured by a strong dye or other coloured substance, such as edicol orange, indigo carmine or charcoal, are placed into the anterior part of the nasal cavity. The time for the dye to appear in the pharyngeal cavity is measured by monitoring its appearance in the pharyngeal cavity [39,148]. Because constant monitoring can be inconvenient for the volunteer, an alternative to the dye method has been developed [144]. In this method,

Table 3
Methods to determine the nasal mucociliary clearance rate in humans

Property	Tracer	Detection	Reference
Total clearance	^{99}Tc-labelled solutions	Gamma-camera	[138–142]
	^{99}Tc-labelled particles	Gamma-camera	[141,159,247]
Mucus flow rate	^{99}Tc-labelled particles	Gamma-camera	[143,144,150]
	^{51}Cr-labelled particles	Gamma-camera	[145]
	Radiopacque Teflon disks	Fluoroscope image intensifier	[146]
Mucociliary transit time	Dye	Pharyngeal inspection	[39,148]
	Saccharin	Sweet taste	[38,41,42,71,139], [144,149–153,179]
	Dye and saccharin	Pharyngeal inspection and sweet taste	[80,155–158]

which has been used in many studies, a sweet-tasting substance, usually saccharin, is deposited in the nasal cavity. The time elapsed between deposition and the sensation of a sweet taste is taken as the mucociliary transit time [38,41,42,71,139,144,149–153]. The disadvantage of the saccharin test is the observation that some subjects have a high taste threshold or do not taste the saccharin [139]. Moreover, performing multiple saccharin tests in a short period of time is impossible, because the sweet taste takes about 4 h to disappear completely [154]. To compensate for the disadvantages of both methods, a combination of a dye and saccharin has been used in several studies [80,155–158].

The outcome of mucociliary transport rate studies can be dependent on whether the tracer is insoluble (e.g. particles, inert dyes, Teflon disks) or soluble (e.g. dye solutions, saccharin). This is due to the structure of the mucus layer, which is composed of the periciliary layer and the outer mucus layer. Insoluble particles will be transported by the mucus layer. Therefore, their transport rate represents the transport rate of the outer mucus layer. In contrast, soluble markers dissolve in both the mucus and the periciliary layer, and their transport rate may reflect the transport rate of both layers [151]. Under normal conditions, both layers are likely to move proportionally and simultaneously in the direction of the beating cilia. In healthy volunteers, an inverse relationship between transport rate and time, i.e. the particle transport rate and saccharin transit time, was observed [150,151]. Furthermore, in volunteers, the saccharin detection time and the clearance half-time of ^{99}Tc-labelled diethylenetriamine pentaacetic acid from the deposition site (Fig. 3) were highly correlated [139]. In some cases, however, the movement of the two layers may be disconnected, resulting in different transit time values obtained with soluble and insoluble tracers [142,146,151,159].

3.4.2. Animal studies

Mucociliary transport studies have also been performed in animals in vivo (Table 2). The nasal mucociliary clearance rate in vivo in rats has been determined with insoluble fluorescent latex particles [160,161]. To investigate the effects of drugs and bioadhesive gels on the mucociliary clearance system, the fluorescent particles were administered nasally, either incorporated into polymer gels, or 30 min after the intranasal application of a drug formulation [160,161]. The nasal mucociliary clearance time was determined by swabbing the oral cavity with cotton tips.

The tracheal mucociliary transport time in rats can be determined in vivo by making a window in the trachea and monitoring the transport of carbon particles [162]. Inter-individual mucociliary transport showed high variability, but within animals, the transport values were consistent. A comparable method was used in rats to determine the nasal mucociliary clearance rate in situ [125]. A window was made to observe the septum, and the tracer material was charcoal. Nasal mucociliary clearance in rats was found to be dependent on sex and on the phase of the oestrus cycle [125]. Based on these results, the use of male animals to determine mucociliary clearance in vivo is recommended.

The ciliary beat frequency in vivo in dogs could be increased by localized surgical incisions [163]. It is therefore important to use a non-traumatizing tissue preparation to determine ciliary beat frequency in vivo. In dogs, such a method has been employed to measure tracheal mucociliary clearance, by using iron particles that were measured with a magnetometer [164].

3.5. Correlation between in vitro and in vivo methods

The nasal mucociliary clearance system is a complex system of interactions among mucus, cilia and the periciliary fluid. Changes in clearance can result both from impairment of ciliary beating (e.g. frequency, amplitude, coordination, or absolute number of cilia and ciliated cells) and from abnormalities in mucus or periciliary fluid (e.g. viscoelasticity or volume). In order to understand and predict the effects that substances can have on the functioning of nasal mucociliary clearance, the results from different in vitro and in vivo models have to be compared and correlated with each other.

The mucociliary wave frequency in humans in vivo does not correlate well with the corresponding ciliary beat frequency in vitro [116]. Moreover, due to the different conditions of measurement, in vitro effects on the ciliary beat may be more pronounced than influences on ciliary activity in vivo [165]. During in vitro ciliary beat frequency measurements,

the ciliated tissue is directly exposed to the compounds investigated. In vivo, the cilia are partly protected by the mucus layer, and the administered compounds will be diluted by the mucus and eliminated by nasal clearance. Furthermore, the epithelial cells in the nasal mucosa are constantly replaced by cells at the basement membrane in vivo. Therefore, the harmful effects of substances on the nasal mucosa may be reversible.

Not only in the case of ciliary activity, but also for mucociliary clearance, a low correlation between in vitro and in vivo methods is observed. The mucociliary clearance rate in vitro in rats, as determined with the frog palate model, did not show any relationship with the mucociliary transport rate in situ [125,166]. A similar observation has been made for the mucociliary transport rate on human nasal turbinates in vitro and the saccharin clearance time in vivo [134]. Reports from studies that try to relate the in vitro nasal ciliary beat frequency with the in vivo nasal mucus transport time are rather controversial. Some studies report a good correlation [167,168], while others reject any relation [42,169]. This discrepancy might be caused by methodological differences and/or intra-individual variations in the mucus transport rate, but also by differences in sensitivity between in vivo and in vitro methods.

Consequently, evaluation of mucus properties or ciliary beat frequency in vitro cannot always be extrapolated to nasal clearance in vivo. In vitro methods are useful for screening compounds with respect to their effect on mucociliary clearance, but the results of these methods are not fully predictive of the ultimate effects on the functioning of the mucociliary clearance system in vivo.

4. Effects of drugs and additives on nasal mucociliary clearance

Many substances can influence the mucociliary clearance system of the airways, either by stimulation or by inhibition. The stimulatory effect of drugs on mucociliary clearance is of clinical importance, because these substances can possibly be used to improve pathological conditions of the mucociliary system [53,75,170–172]. For nasal drug delivery, however, possible inhibitory effects are most relevant, because these effects can result in undesired side-effects on mucociliary clearance. When mucociliary clearance is impaired by components of nasally administered drug formulations, this can prohibit their therapeutic use.

4.1. Drugs

Cholinergic agonists, such as acetylcholine and methacholine, stimulate ciliary activity in a dose-dependent manner in vivo and in vitro, and this effect can be blocked by the cholinergic antagonist, atropine [162,173–177]. Pilocarpine, also a cholinergic agonist, stimulates tracheal and nasal mucociliary transport in vivo in rats, coupled with increased mucus secretion [161,162]. Atropine is the only cholinergic antagonist known to decrease mucociliary clearance and ciliary activity [178].

β-Adrenergic agonists increase ciliary beat frequency in vitro and in vivo [178]. Salmeterol and isoprenaline, β_2-adrenergic receptor agonists, were found to reduce the decrease in ciliary beat frequency of human mucosa in vitro caused by a ciliotoxin [75]. Salmeterol also reduced the decrease in intracellular cAMP and ATP levels caused by the ciliotoxin, and these effects were thought to be mainly mediated by stimulation of β_2-adrenergic receptors [75]. However, salbutamol, also a β_2-adrenergic receptor agonist, was not found to influence nasal mucociliary clearance after intranasal administration in humans [179].

α-Adrenergic receptor agonists, such as xylometazoline and oxymetazoline, inhibit ciliary activity in vitro in several species [80,83,85,89,180,181]. These drugs are used in nasal decongestant sprays for topical application.

Other drugs used in nasal topical applications are corticosteroids and anti-histamine drugs. Nasal sprays of betamethasone with and without neomycin displayed cilioinhibitory effects in vitro, but in vivo, no adverse effects on nasal mucociliary clearance were observed in humans [71]. Corticosteroid sprays also did not cause deleterious effects on the ciliated epithelium of monkeys [182]. The anti-histamine drug, azelastine, resulted in cilioinhibition in vitro in rats, but not in human respiratory tissue [94,171]. Azelastine was not ciliotoxic in animals in vivo [183] and, in patients with allergic rhinitis, it im-

proved the nasal mucociliary clearance rate [184,185].

For a number of drugs that are known to have inhibitory effects on ciliary activity in vitro, the in vivo nasal mucociliary clearance rate was determined in rats [161]. Tripelennamine, lidocaine and bacitracin were shown to decrease ciliary activity in vitro, but clearance rates returned to normal in vivo, except in the case of lidocaine [161]. Lidocaine was also found to cause irreversible ciliostasis of human nasal cilia in vitro, while the ciliostatic effects of 7% cocaine were partially reversible [72]. A 5% cocaine solution has been reported to cause reversible ciliostasis in vitro in chicken embryo trachea at pH 7, while at pH 6, only cilioinhibition was observed [186]. The same pH dependency was found for the effects of 3 and 7% cocaine on chicken embryo tracheal and human sphenoidal ciliary beat frequency in vitro (unpublished results). A 5% cocaine solution resulted in an increase in the mucociliary transit time in chickens [169]. Morphine showed only small cilioinhibitory effects on human nasal and adenoid tissue in vitro, and similar results were obtained for fentanyl and sufentanil [60,187].

General anaesthetics can reduce the mucociliary transport rate in humans, dogs and sheep [188–190]. In vitro exposure to halothane, enflurane and isoflurane caused depression of ciliary beat frequency in human nasal epithelium [56,74]. In monkeys, mucociliary clearance was faster during anaesthesia with ketamine alone, than with both ketamine and barbiturate anaesthesia [191]. Significant differences were found in the nasal absorption of insulin in rats in vivo between rats that were anaesthetized with pentobarbital and Hypnorm, and rats that were only sedated with halothane during dosing or were not sedated at all [192].

A number of peptide and protein drugs investigated for intranasal systemic drug delivery have also been studied with respect to their effects on ciliary activity. Insulin did not cause cilioinhibition in vitro in rats [91] and only resulted in a slight, reversible decrease of the mucociliary transport rate in the frog palate model [131]. Salmon calcitonin had no effects on mucociliary transport in the frog palate model [193], nor on the ciliary beat frequency of mouse septal membranes in culture [103]. Furthermore, human insulin and salmon calcitonin did not decrease the ciliary beat frequency of chicken embryo trachea [86,102].

4.2. Preservatives

Nasal drug formulations for topical or systemic drug delivery usually contain preservatives. These substances have been investigated extensively with respect to their effect on nasal mucociliary clearance and ciliary beat frequency [81,99,100,102,130,139]. Lipophilic preservatives, such as chlorobutol and hydroxybenzoates, caused reversible toxicity to cilia in the presence and absence of mucus in vitro [100,130]. Methyl- and propyl-hydroxybenzoates were also shown to be cilioinhibitory in vitro at concentrations equal to and lower than those in use to preserve aqueous drug formulations [93]. In two studies, Batts et al. [99,130] compared a large number of preservatives at concentrations used to preserve nasal drug formulations, with the frog palate ciliary beat frequency model and in vitro ciliary beat frequency measurements in chicken embryo trachea. The results of both methods did not correlate well. According to the frog palate model, chlorocresol, edetate and benzalkonium chloride caused an irreversible halt of transport; methylhydroxybenzoate, propylhydroxybenzoate and chlorobutol reversibly halted transport; while thiomersal was well tolerated [130]. In vitro ciliary beat frequency measurements showed reversible cilioinhibition for methylhydroxybenzoate, propylhydroxybenzoate and chlorocresol, and irreversible cilioinhibition for chlorobutol. Benzalkonium chloride gave variable results, and thiomersal was more ciliotoxic than benzalkonium chloride [99]. In contrast, in humans, the nasal mucociliary transport time was not altered by benzalkonium chloride, nor by edetate and thiomersal [139].

In a study in which a large number of over-the-counter nasal drug formulations were compared using in vitro chicken embryo trachea ciliary beat frequency, the preservatives were found to play a decisive role in the observed ciliostatic effects [102]. The formulations with the highest ciliostatic effects contained thiomersal and phenylmercuric acetate, and these preservatives were observed to be more ciliostatic than benzalkonium chloride [100]. Menthol and eucalyptol, additives of some nasal deconges-

tants, also displayed strong ciliostatic effects [85,90,102].

Benzalkonium chloride has been shown to cause cilioinhibition and ciliostasis in vitro in chicken embryo tracheas (Fig. 4) [99,100,102] and morphological changes in human adenoid tissue [194]. However, such severe effects on nasal mucosal tissue were not observed in vivo in animals and humans [71,139,182,195,196]. After the long-term administration of corticosteroid formulations containing 0.01–0.02% benzalkonium chloride to rats and monkeys in vivo, no morphological changes of the nasal mucosa were observed [182]. In humans, a corticosteroid nasal spray with 0.02% benzalkonium chloride caused no significant changes in nasal mucociliary clearance after acute and long-term administration. There were also no differences in the ciliary beat frequency before and after the treatment [71]. Furthermore, nasal administration of 0.01% benzalkonium chloride in humans was well tolerated and did not result in changes in the rate of nasal clearance after a single administration [139]. Long-term administration of 0.02% benzalkonium chloride in humans did not change the nasal mucociliary clearance rate, nor did it cause changes in nasal mucosal morphology [196].

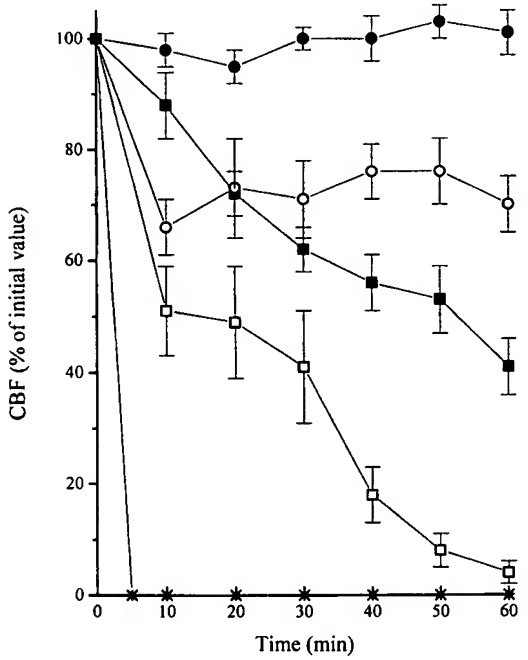

Fig. 4. The effects of additives on the ciliary beat frequency (CBF) of chicken embryo trachea in vitro. Control solutions: Locke-Ringer (– · –) and physiological saline (0.9% NaCl; – ∘ –). Additives: 0.01% benzalkonium chloride (– □ –), 2% methylated β-cyclodextrin (– ■ –) and 1% L-α-lysophosphatidylcholine (– * –). Sodium taurodihydrofusidate (1%) has a similar effect as that of 1% L-α-lysophosphatidylcholine. Data are expressed as the mean±S.E.M. of six–eleven experiments (adapted from [102]).

The effects of decongestant sprays containing preservatives and xylometazoline or oxymetazoline on ciliary beat frequency in vitro showed that the cilioinhibitory effects of the preservative and decongestant are additive [102]. These sprays are available over-the-counter and their long-term abuse is associated with rhinitis medicamentosa, a syndrome of rebound nasal congestion [197]. In rhinitis medicamentosa, epithelial erosion can occur [198], as can a prolonged mucociliary clearance time [172]. Although a benzalkonium chloride-containing decongestant appeared to cause more and longer-lasting rebound decongestion than the same decongestant without preservative [199,200], the inclusion of benzalkonium chloride in nasal decongestants is not a prerequisite for rhinitis medicamentosa [201]. Benzalkonium chloride was found to induce significantly higher rates of mucosal swelling than oxymetazoline, but it did not cause significantly more nasal stuffiness or hyperreactivity [202]. The mucosal swelling caused by benzalkonium chloride explained why the presence of this preservative in a decongestive spray aggravates rhinitis medicamentosa. Benzalkonium chloride was not found to cause changes in nasal morphology or nasal clearance in vivo in other nasal drug formulations [71,182]. Acute and long-term nasal administration of benzalkonium chloride to humans in vivo showed no deleterious effects [139,196]. Therefore, it is not likely that the effects of benzalkonium chloride on mucociliary clearance play a role in rhinitis medicamentosa.

4.3. Nasal absorption enhancers

The absorption efficacy of poorly permeating drugs, such as peptides and proteins, across the nasal mucosa can be improved by the use of absorption enhancers such as bile salts, laureth-9 and fusidate derivatives [203–206]. Sodium taurodihydrofusidate has been found to induce ciliostasis at concentrations of 0.3% and higher, as measured in vitro on human ciliated adenoid tissue using the photoelectric meth-

od. It causes less ciliostasis than 0.3% laureth-9 or 0.3% deoxycholate, whereas glycocholate and taurocholate exert only a mild effect on ciliary activity in vitro [86]. In the frog palate model, full inhibition of the transport rate was observed with 1% L-α-lysophosphatidylcholine, 1% laureth-9, 1% deoxycholate, and 1% sodium taurodihydrofusidate, whereas 1% glycocholate and 1% didecanoyl-L-α-phosphatidylcholine had no substantial effect [131]. The histological effects on animal tissue of these absorption enhancers have been investigated by several research groups, using different contact times. Deoxycholate [207], laureth-9 [208,209], sodium taurodihydrofusidate and L-α-lysophosphatidylcholine [210,211] were all reported to cause severe epithelial disruption (Fig. 5). In human studies of nasal peptide drug formulations, nasal irritation has been reported after single and repeated administration of 1.5% sodium glycocholate [212,213]. In clinical studies with human volunteers, the local tolerability of sodium taurodihydrofusidate was observed to be poor, and it was concluded that its clinical application as a nasal absorption enhancer is undesirable [214].

The absorption of poorly water-soluble lipophilic drugs can be improved by the formation of inclusion complexes with methylated β-cyclodextrins [215,216]. Methylated β-cyclodextrins have also been found to be effective absorption enhancers of peptide and protein drugs [217–220]. In vitro ciliary beat frequency studies of methylated β-cyclodextrins at concentrations of 2 and 4% have shown relatively mild effects on the ciliary beat frequency in vitro, comparable to those of physiological saline solutions (Fig. 4) [102]. Furthermore, the effects of cyclodextrins on ciliary beat frequency were found to be concentration-dependent and reversible [221] Also, studies on the effects of formulations containing methylated β-cyclodextrins and benzalkonium chloride showed that 2% methylated β-cyclodextrin was less cilioinhibitory than 0.01% benzalkonium chloride (Fig. 4) [102]. The effects of both excipients were additive. Methylated β-cyclodextrins showed only mild changes in morphology of the respiratory nasal epithelium in rats, and these effects were smaller than those caused by 0.01% benzalkonium chloride, and were much less severe than those caused by 1% sodium glycocholate and 1% L-α-lysophosphatidylcholine (Fig. 5) [211]. Nasal formulations containing methylated β-cyclodextrins are well tolerated in humans [222,223].

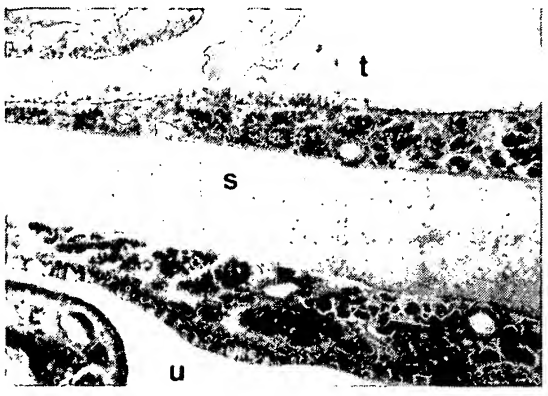

Fig. 5. Rat nasal respiratory epithelium after nasal administration of 1% L-α-lysophosphatidylcholine. Severe epithelial disruption is visible at the treated side of the nasal cavity (100 × enlargement; t, treated side; u, untreated side; s, septum). Adapted from [211].

4.4. Cilioinhibition or ciliotoxicity?

Investigating the effects of drugs and additives on nasal mucociliary functioning is an important issue, because of the increasing number of nasal drug formulations currently being investigated for pharmacotherapy. These preparations are often designed for long-term treatment. Adverse effects on the ciliated epithelium may limit 'patient' acceptance of the nasal formulation and, thus, the use in (sub)-chronic nasal drug delivery.

The measurement of ciliary beat frequency in vitro is a very accurate and reproducible technique to determine the effects on ciliated epithelium [80,84,99,224]. Therefore, ciliary beat frequency measurements are a good in vitro screening method for establishing the potential toxicity of drugs and excipients, and for comparing nasal drug formulations. However, ciliary beat frequency data have to be interpreted carefully, because the effects of nasal formulations in vitro are usually more pronounced than their effects in vivo. The cilia are protected by the mucus layer and the nasal formulations are diluted by the mucus in vivo, whereas in vitro, the cilia are in direct contact with the substances being investigated. Furthermore, in vivo, the respiratory nasal epithelium can be expected to recover from damage. Consequently, it is not possible to make

predictions regarding the effects of chronic use of a formulation on mucociliary clearance in vivo based solely on the effects on ciliary beat frequency in vitro. Nevertheless, substances are often said to be "ciliotoxic" only on the basis of the results of ciliary beat frequency measurements in vitro. The use of the term "ciliotoxic" can be biased, because there are no defined criteria for this classification. Moreover, the cilioinhibitory effects of substances are concentration-dependent and can be additive. These factors also have to be taken into account when determining the toxicity of drug formulations.

There are many cases in which the use of the term "ciliotoxic" is unjustified. For example, the H_1-antagonist azelastine was claimed to be ciliotoxic [225], based on in vitro ciliary beat frequency measurements [94]. However, other ciliary beat frequency studies gave conflicting data on the ciliotoxicity of azelastine, while, in vivo, it was found to be non-ciliotoxic in animals. In allergic rhinitis patients, the long-term use of azelastine even improved the nasal mucociliary transport rate [226]. These findings do not support the classification of azelastine as being ciliotoxic. Classifying substances as being ciliotoxic can be misleading when the concentrations investigated are not in the range of those actually used in nasal drug formulations. For example, in a ciliary beat frequency study, both benzalkonium chloride and chlorobutol were investigated at a concentration of 0.005% [81]. Usually, different concentrations of these preservatives are used, i.e. 0.01% benzalkonium chloride (twice as high as 0.005%) and 0.5% chlorobutol (100 times higher than 0.005%). At a concentration of 0.005%, chlorobutol was observed to be less cilioinhibitory than benzalkonium chloride. Therefore, the wrong conclusion was drawn, i.e. that chlorobutol seemed a safe preservative and that benzalkonium chloride was ciliotoxic [81]. Not surprisingly, this conclusion contradicts the results of a number of other studies with realistic preservative concentrations, in which 0.01% benzalkonium chloride was found to be less cilioinhibitory than 0.5% chlorobutol [99,100,102].

In order to make a preliminary prediction of the effects of substances on nasal mucociliary clearance, not only in vitro ciliary beat frequency studies, but also in vivo studies in animals need to be performed. These can include nasal mucociliary transport rate studies, morphological studies and the determination of the release of marker compounds in the nasal cavity after administration [161,211,227,228]. By studying the effects of nasal absorption enhancers, a good correlation was found between ciliary beat frequency, morphology and the release of marker compounds [211,228]. Based on such studies, the following order of increasing toxicity of nasal absorption enhancers was established: Methylated β-cyclodextrin < sodium glycocholate < sodium taurodihydrofusidate < L - α - lysophosphatidylcholine = laureth-9 = deoxycholate [86,102,211,227,228].

To predict the safety of a nasal drug formulation for human use, it is important to investigate its effects at biopharmaceutically and therapeutically relevant concentrations. Both in vitro and in vivo studies have to be performed, and the investigational formulation has to be compared with nasal formulations that are already used in humans. Moreover, to make accurate and reliable evaluations of the potential side effects of a nasal formulation, the effects of its long-term use in animals and in humans have to be determined. This is essential, because nasal formulations are often designed for subchronic or chronic use.

5. Implications of mucociliary clearance for nasal drug absorption

5.1. Nasal drug absorption

The absorption of drugs from the nasal mucosa is influenced by the contact time between drug and epithelial tissue. Intranasally delivered drugs show a rapid rise to peak blood concentrations [4], and studies in animals and humans have shown that peptide and protein drugs are absorbed by the nasal epithelium within 5–15 min. The "fast" nasal drug absorption is explained by two factors; the permeability of the nasal epithelium and the nasal mucociliary clearance. The first one is the relatively high permeability of the nasal respiratory epithelium for large molecules [1–3], while the diffusion path length through the nasal mucosal epithelium is short, consisting of only two cell layers [5]. The second factor, nasal mucociliary clearance, limits the residence time of drugs administered into the nasal cavity, decreasing the time available for the drug to be absorbed. The normal half-time of clearance in

humans is about 20 min [229,230]. Therefore, strategies to increase the nasal bioavailability of drugs that are poorly absorbed from the nasal mucosa can be aimed either at increasing the nasal membrane permeability, or increasing the contact time for absorption by decreasing the mucociliary clearance rate.

The clearance of a drug formulation from the nasal cavity is influenced by its site of deposition. The turbinates, which are covered by respiratory epithelium in humans, are the primary sites for systemic absorption of nasally administered drugs [231]. Ciliated epithelium is present in the middle and posterior parts of the turbinates, but is almost absent in the anterior regions in the nasal cavity of the rat, where the squamous epithelium is located [211,227]. A drug deposited posteriorly in the nose is cleared more rapidly from the nasal cavity than a drug deposited anteriorly, because mucociliary clearance is slower in the anterior part of the nose than in the more ciliated posterior part [232]. The site of drug deposition in the nose is highly dependent on the dosage form. Nasal sprays deposit drugs more anteriorly than nasal drops, resulting in a slower clearance of sprays than of drops [138]. The nasal absorption of dihydroergotamine in rabbits was significantly higher from sprays than from drops [233]. The nasal bioavailability of the vasopressin analogue, desmopressin, in humans has been demonstrated to be significantly increased following spray administration, compared to nasal drops [138].

5.2. Strategies to increase the residence time of nasally administered drugs

Using a mathematical model that describes the rate processes involved in nasal drug delivery, the effect of bioadhesive carrier systems on the reduction of the mucociliary clearance rate constant can be simulated [234]. The simulations predict that bioadhesion may improve systemic bioavailability, and reduce the variability in nasal drug absorption caused by a variable pattern of drug deposition. The clearance of a nasal preparation from the nasal cavity may also be influenced by the viscosity of the preparation. The rheological characteristics of the polymers determine their ability to reduce the mucus transport rates, but not their chemical structure [135]. Many hydrophilic macromolecular materials, including guaran, polyacrylamide, agarose and gelatin, which are chemically quite dissimilar to mucus, are capable of being transported on a mucus-depleted frog palate [118]. It is possible to predict the optimal rheological properties that a polymeric formulation must possess in order to minimize its clearance by the mucociliary transport system [127,128].

Spray preparations containing 0.25% methylcellulose have been reported to exhibit decreased mucociliary clearance [235], resulting in a delayed absorption of nasally administered desmopressin, without affecting the bioavailability of desmopressin [236]. The clearance half-time of nasal spray solutions containing hydroxypropyl methylcellulose tended to increase with increasing concentration, but the differences between the concentrations were not significant [237]. Studies on the nasal delivery of beclomethasone, formulated in a powder mixture with hydroxypropylcellulose as a bioadhesive, have shown that the formulation remains in the nasal cavity for as long as 6 h after application, with apparently no damage to the nasal mucosa [238].

A number of mucoadhesive delivery systems have been investigated for intranasal drug administration. Freeze-dried formulations of insulin and neutralized polyacrylic acid increased the nasal absorption of insulin in dogs, and the absorption was sustained with maximal plasma insulin levels 90 min (T_{max}) after administration [239]. Hyaluronan and its autocross-linked esters showed good adhesion in vitro, comparable with that of polyacrylic acid, but microspheres of hyaluronan and its autocross-linked esters were less adhesive [129]. Although the formulation of hyaluronan into microspheres tended to reduce its adhesive properties, the microspheres displayed significantly decreased mucociliary clearance on the frog palate [129].

The adhesion of water-soluble polymer powders to the nasal mucosa was investigated both in vitro and in vivo [240]. The rank order of adhesion of the polymers to agar plates in two in vitro methods appeared to be quite similar to that of their mucoadhesion in vivo. This was measured by observing a dye mixed with the polymer after its application to the nasal cavity of a rabbit, using a thin fibrescope. Xantan gum showed the longest residence time in the nasal cavity, followed by tamarind gum, hydroxypropylcellulose and polyvinyl alcohol [240]. Several bioadhesive polymers were

compared with respect to their nasal mucociliary clearance rates in rats in vivo [160]. The decrease in clearance was the largest for 3% methylcellulose, and the smallest decrease was found for 0.2% polyacrylic acid. However, it was observed that the total clearance of a polymer gel formulation was not dependent on its initial clearance. Very viscous or fluid formulations demonstrated rapid initial bulk clearance, but if they were bioadhesive, their total clearance from the nasal cavity was limited [160].

In order to reduce nasal clearance and thereby increase nasal drug absorption, microspheres have been studied as nasal dosage forms [141] Albumin, starch and DEAE–Sephadex microspheres, with a diameter of 40–50 μm, appeared to have clearance half-life values of 3 h or more, compared to 15 min for solutions and powder formulations [141]. These remarkably reduced clearance times are probably caused by swelling of the microspheres, thereby forming a mucoadhesive intranasal delivery system. Degradable starch microspheres considerably increased the bioavailability of nasally administered insulin, desmopressin and gentamicin in rats and sheep [241–243]. Hyaluronic ester microspheres also increased the nasal absorption of insulin in sheep [244]. However, the rate of absorption of these drugs after intranasal administration in microspheres in sheep was not delayed, because the T_{max} values were small, ranging from 8 to 34 min [241,243–245]. In the case of the absorption of insulin in sheep, only a slight increase in T_{max} values was observed, from 5–8 min for solutions to 12–22 min for microspheres [243]. The nasal absorption of desmopressin from microspheres in sheep also showed only a slight increase or even a decrease in T_{max}, compared to that of solutions [245]. After intranasal administration of insulin in starch microspheres in rats, a rapid decrease in blood glucose was also observed [246].

Apparently, the enhancing effects of microspheres on nasal drug absorption are not caused by a reduction in mucociliary clearance. This is supported by the finding that bioadhesive starch microspheres have not been shown to affect mucociliary clearance in humans [155,246]. Studying the effect of posture on the nasal clearance of bioadhesive starch microspheres, they were found to be retained significantly longer than were solutions [247]. However, the prolonged retention appeared to occur primarily in the anterior non-ciliated region, and not in the absorptive respiratory regions of the nasal cavity. Furthermore, ^{99}Tc-labelled microspheres were mainly deposited in the nasal vestibule [247]. This deposition pattern can explain the lack of effect of microspheres on nasal mucociliary clearance. Microspheres probably increase nasal drug absorption by acting as absorption enhancers, possibly by transiently opening the tight junctions of the nasal mucosa [246,248].

Chitosans have been proposed as novel nasal drug delivery systems because of their bioadhesive properties. In sheep, the nasal absorption of insulin was increased after the administration of chitosan, and the T_{max} was delayed from 20 min for a solution of insulin, to 75 min for a solution of 0.5% chitosan with insulin [249]. The morphology of the nasal mucosa of rats was not changed after application of a chitosan solution for 60 min [249]. Frog palate mucus clearance was transiently decreased by chitosan [133]. The reduction of mucociliary clearance on the frog palate was found to depend on the molecular weight of the chitosans, possibly because the longer chitosan chains are more entangled with the mucus network and therefore more mucoadhesive [133]. However, after insulin administration with chitosans of different molecular weights, no significant differences were found for the drop in blood glucose levels in rats [250]. In rats, only a small amount of a 3% chitosan gel was cleared from the nasal cavity within 2 h [160].

From all of these studies with bioadhesive formulations, it is clear that they cause absorption enhancement, but additional evidence is needed to conclude that the improved absorption in vivo is mainly caused by a prolonged residence time.

6. Conclusions

The function of mucociliary clearance is to protect the nose and the lower airways from damage by inhaled noxious substances. The impairment of the mucociliary clearance system is related to the occurrence of respiratory diseases. It is therefore of great importance to determine the effects of nasal drug delivery systems on nasal mucociliary clearance, because impairment of mucociliary clearance by these systems can be prohibitive of their therapeutic use.

Since nasal mucociliary clearance is a complex system of interactions between mucus, cilia and the periciliary fluid, a large number of models exist to investigate the effects of substances on the mucociliary system. The results obtained with these models do not always correlate well, not only for the different in vitro models, but also for in vivo versus in vitro models. The in vitro methods are useful for screening compounds. Ciliary beat frequency measurements have been shown to be a good indicator of the effects of substances on nasal tissue morphology. Therefore, they are valuable tools in the design of nasal drug formulations. However, to determine the in vivo ciliotoxicity of compounds, it is not reliable to use only in vitro methods.

In the development of novel peptide and protein formulations for nasal drug delivery, the potential side-effects on nasal mucociliary clearance need to be investigated at an early stage. Adverse effects on mucociliary clearance will limit a patient's acceptance of the nasal formulations and their use in nasal drug delivery. In particular, the effects on mucociliary clearance of nasal absorption enhancers and preservatives need to be investigated, because some of these substances exhibit mild-to-severe cilioinhibition in vitro.

Another aspect of the effects of nasal drug formulations on nasal mucociliary clearance is the possibility of increasing nasal drug absorption by transiently decreasing the mucociliary clearance rate. A prolonged residence time in the nasal cavity can be achieved by using bioadhesive polymers, microspheres and chitosans, and by increasing the viscosity of the formulation. However, the prolonged retention of microspheres appeared to occur primarily in the anterior non-ciliated region, and not in the absorptive respiratory regions of the nasal cavity. Therefore, the absorption rate of drugs administered in bioadhesive formulations is not always delayed in vivo. Further evidence is needed to warrant the conclusion that the increased bioavailability of nasal bioadhesive drug formulations is primarily caused by a longer residence time of the formulation in the nasal cavity.

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EXHIBIT F

"Measurement of Nasal Mucociliary Clearance,"
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Measurement of nasal mucociliary clearance

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The International Symposium on the Nose and Bronchi offers the opportunity to, on one hand, view the respiratory tract as a whole, and, on the other hand, to look for significant differences between the upper airways and those of the lungs. Outstanding among such differences are the vascularity and secretory apparatus of the nose and their adjustability to ambient demands, and perhaps some differences in mucociliary clearance. This paper is addressed to the measurement of the latter function in man.

After Hilding's brief but excellent report in 1931 (5) on nasal clearance currents, innumerable studies were done using visible particles as tracers (Table 1). In order to provide an alternative method which would permit study of portions of the nasal passage not readily accessible to vision and accurate measurement of clearance rates also many methods using invisible tracers have been developed (Tabel 2) Quinlan et al (7) developed the technique which we have found most useful. This made use of a small inert particle, tagged with Technetium, the movement of which could be measured by external detection equipment The method has been described in detail elsewhere (1-3). Its essential points are:

1. The particle employed is not soluble in airway secretions.
2. Its radioactive tag is of short half life and relatively innocuous in the dose of radiation it delivers (we generally use a tag of about 3 μc).
3. It is gently placed on the superior surface of the inferior turbinate approximately 1 5 to 2.5 cm posterior to the anterior turbinate tip (this is in the path of the main stream of inspiratory air flow).
4. The head of the subject is immobilized in respect to the external detection apparatus.
5. In addition to the collimated detectors which view along lines crossing the nasal passage laterally, there is an uncollimated detector in front of the nose yielding a record of anterior-posterior motion.

The question has been raised as to whether the clearance rate of such a particle truly reflects the rate with smaller, naturally occurring airborne materials. It has been repeatedly shown that the size or weight of a particle (within reasonable limits) does not significantly affect clearance rate (8); and we have had the opportunity of observing in the dog's trachea the similarity in clearance of this and other types of materials.

A second technique was developed by us for use in circumstances when the radioactive method was not available or desirable. It involves placement of a similar particle with a strong taste in the same spot and noting the time which elapses before the

Table 1. Methods for measurement of human nasal mucous flow rate with visible tracers

Author	Year	Tracer	Detection method
Yates	1924	dye solution	direct observation
Hilding	1931	india ink	direct observation
Freckner	1939	small piece of paper	direct observation
Ornston	1946	sulfathiazole powder	posterior rhinoscopy
Tremple	1948	edicol orange powder	posterior rhinoscopy
Van Ree & Van Dishoeck	1962	edicol orange powder	posterior rhinoscopy
Bablik	1965	chalk and coal dust	pharyngeal inspection
Ewert	1965	edicol supra orange	anterior inspection by binocular operating microscope
Bang, Mukherje & Bang	1967	sky blue solution	pharyngeal inspection
Andersen, Camner, Jensen, Philipson & Proctor	1974	saccharine	report of sweet taste
Andersen, Andersen & Solgård	1977	saccharine/dye	report of sweet taste, then pharyngeal inspection

Table 2. Methods for measurement of human nasal mucous flow with invisible tracers

Author	Year	Tracer	Detection method
Proctor & Wagner	1965	microliter drops of I 131 tagged albumen	(1) serial nasal scans (2) two-slit collimated scintillation detectors
Quinlan, Salman, Swift, Wagner & Proctor	1969	Tc 99^m tagged resin particle	gamma camera
Lippman	1970	Nasal inhalation of Fe tagged aerosol	Nasal retention by scintillation detectors
Andersen, Proctor et al	1971	Tc 99^m tagged resin particle	six-slit collimated scintillation detector
Black, Evans, Hadfield, Macbeth, Morgan & Walsh	1973	microdrop Tc 99 m tagged suspension of polystyrene	two-slit collimated scintillation detector
Sackner, Epstein & Wanner	1977	radiopaque teflon mini discs	fluoroscope image intensifier and videotape

subject notes the taste (we have used saccharine). This depends upon a subjective response; but the particle can be colored with a dye, the appearance of which in the pharynx coinciding with the report of the taste confirms the passage. The normal time for clearance is about 12 to 15 minutes. Anything over 30 minutes is considered abnormal.

A disadvantage of this method is that saccharine is soluble and it may be cleared in periciliary fluid under circumstances when surface particle clearance is impeded. So far

we have found in normal subjects that rates measured by the two methods correlate well with one another

In the study of several hundred relatively normal adult subjects our main findings have been:

1. The average rate of nasal clearance is about 8 mm/min, and the range is between less than 1 and more than 20 mm/min.
2. About 20% of subjects exhibit very slow clearance rates, under 2 mm/min.
3. We have not found the same similarity between nasal clearance in monozygotic twins as Camner found in bronchi, and there was no good correlation between nasal and bronchial clearance in the same subject.
4. There is a region in the anterior nose where clearance is forward.
5. Ambient relative humidity between 9 and 70% (at 23°C) has no effect on nasal clearance.
6. Ambient air temperature change from 23°C to temperatures between 7 and 39°C produced minor transitory falls in clearance rate.
7. SO_2 at 4 ppm slows nasal clearance in the anterior nasal passage, and formaldehyde at 0.3 mg/m^3 has a similar effect.
8. Occupational exposure to hardwood dust also slows clearance in the anterior nose
9. An inert dust, even at 25 mg/m^3 has no effect upon clearance, and the same is true of toluene at 100 ppm.
10. A recent study has shown a reduction in nasal clearance among workers in grain silos

Comprehensive descriptions of these findings are given in (4) and (6).

There is ample evidence that mucociliary clearance in the major bronchi and trachea are similar in rate and mechanism to nasal clearance Clearance in the small airways of the lungs, where mucus secretion is absent or sparse, is an entirely different matter. But that there are significant differences between the nose and bronchi is clear from the findings mentioned in items 2 and 3 above, that a large number of apparently healthy young subjects exhibit very slow nasal clearance, that the genetic influence found for bronchial clearance is not so evident in the nose, and that bronchial and nasal clearance do not correlate well in the same individual. It would be important to know whether ciliary abnormalities, differences in airway secretions, or some other factor account for this difference We have suggested the possibility that mucosal injury during childhood from viral infections and/or ambient air exposure may account for this. That hypothesis could be tested by appropriate studies in children.

In conclusion we should point out that a method of studying clearance, both nasal and bronchial, which would clearly differentiate between surface mucus clearance and clearance in periciliary fluid needs to be developed.

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DISCUSSION

Dr Newhouse had tried to compare the saccharine test with the radio-isotope test and had found that both the sensitivity and specificity of the saccharine test was low. He had therefore abandoned this method *Dr Andersen* replied that it is of crucial importance to position the particle at exactly the right place. He agreed that there was a very large interindividual variation but said that this also applied to other methods. *Dr Mygind* added that the reproducibility of the test could be increased by placing a particle on both sides of the nose. It is also important to check that the patients are not sniffing, blowing their noses or have too much watery rhinorrhoea In his opinion this is a valuable method as it is the only test which can be carried out outside the research laboratory repeatedly and easily. *Dr Puchelle* emphasized the importance of placing a particle on both sides as they had found a marked influence on the results from the nasal cycle The transportation time was two-fold lower on the congested side than on the open side